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FROM THE

LABORATORY OF GENETICS

Vol. III

BOSTON
1920-1928

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CONTENTS

Population. E. M. East.
Genetic Studies on the Protein Content of Maize. E. M. East and D. F. Jones.
A Study of Partial Sterility in Certain Hybrids. E. M. East.
The Agricultural Limits of our Population. E. M. East.
Round Tip Tobacco—A Plant "Made to Order." E. M. East and D. F. Jones.
As Genetics Comes of Age. E. M. East.
Sterility in Wheat Hybrids. II. Chromosome Behavior in Partially Sterile Hybrids. Karl Sax.
Sterility in Wheat Hybrids. III. Endosperm Development and F_2 Sterility. Karl Sax.
Mendel and His Contemporaries. E. M. East.
Population in Relation to Agriculture. E. M. East.
Genetics of Bunt Resistance in Wheat. E. F. Gaines.
Studies on Self-Sterility. VI. The Genetic Basis of Cross-Sterility in Nicotiana. Edgar Anderson.
Genetical Aspects of Self-and Cross-Sterility. E. M. East.
The Physiology of Pollen. R. A. Brink.
Meiotic Cytokinesis of Cannabis. Hugh C. McPhee.
The Influence of Environment on Sex in Hemp (Cannabis Sativa L. Hugh C. McPhee.
A New Interpretation of the Hereditary Behavior of Self-Sterile Plants. E. M. East and A. J. Mangelsdorf.
Studies on Self-Sterility. VII. Heredity and Selective Pollen-Tube Growth. E. M. East and A. J. Mangelsdorf.
Linkage between a Flower Color Factor and Self-Sterility Factors. Friedrich G. Brieger and A. J. Mangelsdorf.
The Physiology of Self-Sterility in Plants. E. M. East.
Color and Sex in the Indian Walking Stick, *Dixippus Morosus*. A. J. Mangelsdorf.
Mendelian Factors Producing Selective Fertilization. Friedrich G. Brieger.
The Expression of Mendelian Factors in the Gametophyte of Maize. P. C. Mangelsdorf and D. F. Jones.
Cytological and Genetic Studies on Fragaria. K. Ichijima.

Über genetische Pseudofertilität bei der selbststerilen Nicotiana Sanderæ hort. Friedrich Brieger.

Inheritance of Trimorphism in Lythrum Salicaria. E. M. East.

Pollen-Tube Growth in Lythrum Salicaria. Dontcho Kostoff.

Origin of the Garden Strawberry. A. J. Mangelsdorf.

Studies on the Genetics of Fragaria. A. J. Mangelsdorf and E. M. East.

The Genetics and Physiology of Self-Sterility in Nicotiana. E. M. East and A. J. Mangelsdorf.

The Inheritance of Heterostyly in Lythrum Salicaria. E. M. East.

The Growth and Development of Plastids in Lunularia Vulgaris, Elodea Canadensis, and Zea Mays. Conway Zirkle.

Induced Immunity in Plants. Dontcho Kostoff.

Cytological Studies in the Genus Nicotiana. M. Christoff.

The Effect of Hydrogen-Ion Concentration upon the Fixation Image of Various Salts of Chromium. Conway Zirkle.

The Genetics of Trimorphism in Lythrum Salicaria. E. M. East.

Heredity in the Genus Fragaria with Special Reference to the False Hybrids of Millardet. E. M. East.

The Genetics of the Genus Nicotiana. E. M. East.

POPULATION

By Professor E. M. EAST

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[Reprinted from THE SCIENTIFIC MONTHLY, June, 1920.]

POPULATION[1]

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THERE is scarcely a subject in whose depths one may escape the problem of population. It pervades history and sociology, it penetrates ethics and philosophy, it influences science and the arts. This is because it is solely in man's own interest, his food and protection, his love and happiness, that he founds social and political systems, establishes codes of morals, and otherwise busies himself going to and fro upon the earth. If there be an unvoiced criticism that these are mere platitudes founded on man's domination of the world, and that a diversity of phases in the problem of population, as usually delimited, does not necessarily follow, it is not well-founded. The *problem* of population takes form when it is realized what a large number of these vital interests of mankind are antagonistic to each other, are even mutually exclusive. And as a problem not one of these numerous ramifications may profitably be excluded.

In spite of the catholicity of the subject, it has had definite consideration by only two classes of thinkers, theologians and economists. The theologians have discussed it with their habitual effusiveness, but their examination has been superficial. For them, mundane happiness has a very low order of magnitude. It is a distasteful matter, unworthy of the spiritually minded. Such progress as has been made, and it should not be minimized, has resulted from the analysis of the economists. And I say this mindful that the man who made the greatest original contribution to the subject was entitled to place the word Reverend before his name; for he wrote as an economist, not as a theologist.

I submit, however, that there should be no such monopoly. Such a momentous and difficult problem should not be the sole property either of priest or politician, of economist or social worker. I do not suggest its preemption by the biologist; yet by its nature the question is fundamentally biological, and if the biologists do not interest themselves in it, it can not receive the attention it deserves.

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[1] Address of the retiring President, delivered at the thirty-seventh annual meeting of The American Society of Naturalists held at Princeton, New Jersey, December 30, 1919.

The problem of population is not new. Like all general questions it has interested mankind ever since there has been some modicum of civilization. But, with that scholasticism characteristic of the majority of publicists of all times, it seldom has seemed necessary to base their statements, theories, or laws, upon concrete facts. One obtains little reward other than cynical amusement in following out its history, from the *Lex Papia et Poppœa,*[2] enacted for the purpose of encouraging marriage and legitimate fecundity, by the efforts of two old bachelor consuls in the year 10 A.D., to the analogous contemporary propaganda of our erstwhile bachelor colleague Popenoe,[3] from the naïve teachings of the early Christian fathers, to the modern headline that "Mrs. Blindly Helpful warns of birth decline,"—probably in the same awed tone that Little Orphan Annie whispered, "the goblins 'ill git you." But even as the development of the doctrine of evolution is comparatively uninteresting until one reaches Lamarck and Darwin, the theories of population have little intellectual standing until the time of Franklin[4] (1751) and Malthus[5] (1798).

Franklin's chief contribution to the subject was a short essay entitled "Observations concerning the Increase of Mankind and the Peopling of Countries." In it the dependence of population increase or decrease on food, commerce, type of government and conditions of labor, were succinctly and accurately stated. Malthus acknowledged his indebtedness to Franklin; but the fate of Franklin's essay as compared to Malthus's treatise was as those of Wallace and of Darwin on Natural Selection, the logic was good but the basis of material fact was small.

Malthus, on the other hand, delved successfully for facts and found their meaning. In spite of bitter criticism his work is accepted to-day by leading economists with little change. The only difficulty is that the world is inclined to blunder along without heeding Malthus's warning. It sits gaily at its present feast oblivious of the *mene tekel* on the wall. How many of our leading biologists, even, have read the "Essay on Population"? They know it gave to Darwin his long-sought concrete cause of organic evolution, Natural Selection, but in general can they do more than speak vaguely of a geometrically increasing population pressing upon an arithmetically increasing food supply? If the scholar thus casually passes by a fundamental cause of past, present and future social unrest, can one justly call to court the harebrained Bolshevist for his fallacious doctrines, or rail against legislators, who, true to their primary principle of holding firmly to their jobs, pass resolutions to investigate the high cost of living or the relations of labor and capital, with the same aplomb as they would undertake to investigate the law of gravitation could such a procedure further their political fortunes?

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[2] Strangeland, C. E., "Premalthusian Doctrines of Population," Studies in History, Economics and Public Law, Vol. 21, No. 3, pp. 1–356, 1904.

[3] Popenoe, P. and Johnson, R. H., "Applied Eugenics," New York, Macmillan, 1918, pp. 459.

[4] Franklin, B., Miscellany. (1751.)

[5] Malthus, J. R., "An Essay on Population," 3 vol. Georgetown, Milligan, 1809. First American from third London edition.

Malthus's work went through many editions. In some particulars the first imprint was more pessimistic than the later revisions. Perhaps if the author were alive to-day he would return to his first impressions; but since the purpose of this paper is to look into the facts as they affect the present and the immediate future, I shall epitomize his mature conclusions as given in the third edition.

The object of his inquiry was to examine the causes impeding the happiness of mankind, and to speculate on the probability of their removal. The chief cause of distress and misery he attributed to the constant tendency of man, in common with the lower animals, to increase beyond the means of subsistence. Irrational animals, he states, are powerfully impelled to increase their species freely, deterred by no doubts about providing for their offspring; and the results of this freedom are afterwards repressed by want of room and nourishment. Mankind, impelled to increase by the same instinct, is somewhat checked by reason; but nevertheless the tendency is such that the increase of mankind actually does press upon the means of subsistence to such an extent that various forms of misery, or the fear of misery, are the direct result.

The ultimate check to population is thus a want of food which necessarily results from the different ratios according to which population and food increase.

> The immediate check may be stated to consist of all those customs, and all those diseases, which seem to be generated by a scarcity of the means of subsistence; and all those causes, independent of this scarcity, whether of a moral or physical nature, which tend prematurely to weaken and destroy the human frame.

These obstacles to increase, he maintained, were resolvable into moral restraint,—to which one may to-day add artificial family limitation,—vice, and misery. To state the matter a little differently, increase in population is the chief cause of misery in so far as that misery is caused by the struggle for existence. Under this head he named "unwholesome occupations, severe labor, extreme poverty, undernourishment of children, great towns, excesses of all kinds, the whole train of common diseases, wars, plagues and famine."

One may without exaggeration add materially to this list, for all of man's activities are resolvable into a struggle for existence and a struggle for perpetuation, and it is the antagonism of these two instincts that leads to all the trouble.

To my mind, the Reverend Doctor proved his case very neatly in the three volumes of his work. Not only that, but he discovered two principles as corollaries to this main thesis, which are particularly important at the present day. The first is to the effect that emigration relieves population but temporarily, owing to the increased birth rate of the remaining population resulting from a release of the economic pressure. The second is that the lower classes of a population tend to replace the upper classes,—a natural conclusion following from the fact that that part of the population which takes no thought of the future has the highest birth rate.

Nevertheless, in spite of this thorough and scholarly attempt to place before the public logical conclusions on this great problem, induced from concrete facts, in spite of later analysis and acceptance by every economist of note, there is still a hue and cry for population. European countries, crowded to the utmost, appoint commissions to inquire into their declining birth rate, and to take steps to increase it. Tempestuous people in the public eye thunder forth *dicta* about the subject, though blindly ignorant of the facts and making no pretense of learning them. Even those who pass for scientists and might be expected to look into the subject, seem obsessed with the same idea. Witness a paper[6] delivered a short time ago by the statistician of the Metropolitan Life Insurance Company at a meeting of the American Association for Advancement of Science, and afterwards spread broadcast as a part of *The Congressional Record.* It is difficult to account for such narrowness of view, superficiality of logic, and general inability to grasp the broad problem, as is exhibited in this paper. If these are the reactions of those from whom some basis of knowledge might reasonably be demanded, what is to be expected from the man on the street who is entranced as with a toy by the mere idea of bigness—a big country, lots of people—let them live as best they may. But enough of this; let us examine the facts on the subject as we face them to-day.

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[6] Dublin, L. I., "The Significance of the Declining Birth Rate," *Science*, N. S., 47: 201–210, 1918. See also reply with same title by Ellen Hayes, *Science*, N. S., 50: 533–536, 1919.

In the time of Malthus, the world could still be considered as a collection of more or less isolated geographical entities. Succeeding the original development of subraces through real isolation, there had been waves of migration with resulting intermixtures of peoples, it is true; but at that time, a short 100 years ago, there remained a condition of geographical separation which has now been swept away. The world war brought home to us the illusiveness of distance. In considering the question before us from the broadest point of view, the whole globe must be treated as a unit.

How fast the population of the earth has increased in the past is an unknown quantity, and will remain unknown. An estimate of the present population or of its natural increase is not accurate by any means, but is more than a random conjecture. From the returns of the Registrar General of England, the Census reports of the various civilized countries, the Statemen's Year Book, and the opinions of several travellers in Africa and the Orient, I estimate that there are at present 1,700 million people. A careful review of the factors involved leads me to believe that the probable error is not greater than ± 40 million. Again, the annual natural increase estimated country by country, disregarding the effect of the war, totals not less than 14 million or more than 16 million. This is an average rate of about 9 per thousand. To show that this estimate is conservative, I may say that a letter from the Office of the United States Census rates the annual increase of population in the world at approximately 25 million.

Consider a moment what these figures mean. Not long ago we were asked to speed production, to save, to waste nothing, that Belgium might be fed and clothed. We did this and more, and we may well be proud of a difficult task efficiently accomplished. But, have we realized, can we realize, that 2 Belgiums are added to the world's population each year? And all must be fed,—though perhaps some need not be clothed.

Segregating this increase by races as accurately as one may, shows that the white race is increasing much more rapidly than either the yellow or the black. China's 300 million population is practically stationary; India and the South Sea Islands are increasing spasmodically, but probably not at a greater rate than 8 per thousand. Japan, on the other hand, has a natural increase of over 13 per thousand; and the theoretical curve fitted to the crude birth rate is *rising*, though the crude death rate is stationary. The blacks are increasing rapidly only in this country, where the natural increase is now about 11 per thousand, and in the West Indies. In Africa they are increasing but slowly. In some parts they are actually decreasing, and it is doubtful if they reach the figure for the United States even in the richest sections of the continent. The white race is the race on the road to numerical domination. With the exception of France, few white peoples are increasing at a less rate than 10 per thousand. The countries of eastern Europe, Russia, Rumania, Bulgaria and Serbia, with tremendous birth rates, from 40 to 50 per thousand, are increasing at a rate of from 17 to 19 per thousand, while Australia and New Zealand, with low birth rates, from 26 to 28 per thousand, are multiplying at nearly the same speed, due to their low death rates.

It is natural to ask how the Great War affected these estimates. No precise answer to the question can be given, because it is just possible that the after effects of this war will be unlike the after effects of previous wars, in that the birth rate will continue to be depressed below the pre-war figures. Personally, I do not believe this will be the case. I believe we may read the future in the past, and that the birth rates will take such upward trends in the war stricken countries that in a very few years they will readily fit projections of the pre-war curves. In other words, I believe it to be a fair prediction that so far as general gross natural increase of population is concerned, the curve when plotted from 1900 to 1950, if that be some day possible, will show only a temporary deflection for the years 1914 to 1919. The tide of population is not kept back by the flimsy barrier of war, it is but baffled for the moment.

Naturally, if one looks at the subject more minutely, he cannot deny the great influence of the war upon the countries primarily involved. The reports are conflicting, but various official estimates place the direct losses at between 10 and 12 million. The indirect losses are hardly more than half as much, —perhaps much less. Indeed if one could correct for lessened death rates due to army training, the total would be materially diminished. It may be doubted, therefore, whether all losses, direct and indirect, attributable to the war, are over 18 million. And I may say that this statement is not made haphazard, but after due consideration of the data from the statistical divisions of several of our own and other governmental agencies. Compare this, then, with the effects of the pandemic of influenza in which not less than 20 million people are thought to have lost their lives. Numerically, both of these figures are terrible, staggering, incomprehensible; yet on a percentage basis they are trifling when compared with the wars and plagues which swept Europe and Asia in former times, and from which they recovered with astonishing rapidity.

Let us turn for a moment to the potential loss due to the decrease in the birth rate. An estimate in 1917,[7] based on cities in Germany comprising about one sixth of the population, showed that the German birth rate had dropped about 10 per thousand. If we assume this to be correct for warring Europe plus Turkey in Asia, three years potential loss is approximately 8,500,000.[8] This figure allows Russia in Europe but one third the loss of the others, for the very good reason that the Russian manner of living is such that a loss of 3 or 4 per thousand more nearly represents the facts, and excludes the United Kingdom where the depression was slight. This again is a great potential loss, but is it not small compared with the total population of the countries involved, and will it not be made up as it has at the close of all other wars by a temporary supernormal birth rate? Furthermore, in central Europe it was found that the war conditions actually made for a lower infant mortality, probably because of a greater frequency of enforced breast feeding; and the actuality of the potential loss is not wholly what it seems to be.

These facts are given for the purpose of showing that in spite of wars, notwithstanding plagues and pestilences, the world goes merrily on obeying to the letter the biblical injunction, "increase and multiply." But I hear the question, is this going to continue? Is there not a generally decreasing birth rate? Yes, this is true. In most of the civilized countries of the world the birth rate is slowly but steadily decreasing. The result, however, is not what many would have us believe. The inter-nation correlation between the birth rate and death rate is high. In general, where the birth rate is high, the death rate is likewise high.[9] Where the birth rate is low, the death rate is low. Australia, New Zealand and Holland with low birth rates have tremendous rates of natural increase, rates much higher than many other countries with very much larger birth rates. There is but one outstanding exception to this rule—France. Though France reduced her births, she failed to reduce her deaths as fast as might reasonably be expected in a country with her standard of culture. Having reached a point where there is practically no natural increase, therefore, her death rate more nearly measures the absolute length of life of her inhabitants, and for that reason seems abnormally high. Were the birth rate of France to take a sudden rise, there would be the apparent paradox of a fall in the death rate due to an increasing population. Nevertheless France stands alone in this. Other countries from which we have statistics are still increasing, and in them the positive correlation between the birth and death rate holds; although in saying this we make no claim as to the relation for intra-nation correlation on a time basis, for the simple reason that it has not been adequately studied. It is a fair prediction, however, to say that owing to the steadily increasing development of medicine and of general sanitation, the decrease in the birth rate will have no great effect on the natural increase in the world for many years to come. In fact it would not be surprising if during the next 50 or 100 years the excess of births over deaths should go up rather than down in many countries.

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[7] Vital Statistics in Germany. Taken from Veröffentl. d. k. Gsndhtsamtes (41: 1917), by War Office. Issued in Medical Supplement, Review of the Foreign Press.

[8] *Cf.* Mallet, Sir Bernard, "Vital Statistics as Affected by the War," *Jour. Roy. Stat. Soc.*, 81: 1–36, 1918.

[9] *Cf.* Drysdale, C. V., "The Small Family System," New York, Huebsch, 1917, pp. 196. Also "The Declining Birth Rate: its Causes and Effects." Rpt. National Birth Rate Commission (Great Britain). New York, Dutton, no date (preface dated 1916), pp. 450.

What is to become of this flood of people? The international situation at present is this. China is stationary in population,—a high birth rate (if we may believe reports), and a high death rate. With a permanent system of agriculture she feeds herself. Northern Asia, Central Asia and even India can support a few more people. Australia and New Zealand are increasing at a rate which their possibilities in the way of food production can stand for only a short time. Europe, as a whole, is already over-populated. England is in the least desirable condition, with the countries of northern Europe running her a close second. By great efforts Europe can support its present population without extreme hardship, but the efforts must be sustained and efficient. There remains then, Africa and South America, as colonization centers,—the United States we leave for separate consideration. These places should be able to support a large number of people. True, large portions are tropical, and the white man has not been a particularly successful occupant of the tropics; nevertheless one may predict, without undue optimism, that these difficulties will be conquered, and that these lands will repeat the history of North America. There will be emigration from Europe, and perhaps from Asia; there will be a birth release in the new lands; and they will teem with people. And let us make no mistake here. If science makes this development possible, the time when Africa and South America are filled to the practical limits of their food production is no dim and distant future. If the rate of increase actually existent during the nineteenth century in the United States should obtain, within the span of life of the grandchildren of persons now living, these countries will contain over a billion inhabitants.

Long before this eventuality, the struggle for existence in those portions of the world at present more densely populated will be something beyond the imagination of those of us who have lived in a time of plenty. Each geographical unit must then of necessity produce its food. It will be impossible for a country to maintain a position such as that of England. Excess population supported by commerce will be a thing of the past. There will be commerce, of course, but exportation of food in quantity will not longer be tolerated.

To present this matter in a way which will bring home the economic and biological consequences of population pressure, let us consider it in some detail as it affects the United States. We have taken the census thirteen times,[10] 120 years,—not a long period as even history is measured. In 1790 the population was 4 million; in 1910 it was 92 million. In 1920 we may expect to have somewhere near 110 million people. In 120 years, the country had increased in population 23 times; in 130 years, it presumably will have increased 27 times. More minutely, of the 92 million returned by the enumerators in 1910, 82 million were white and 10 million yellow and black,—chiefly the latter. But of the 82 million white, only 68 million were native white, over 13 million being foreign born. If we look a little closer, we find that only about 49 million were native whites of native parentage,—32 million being either foreign born or of foreign or mixed parentage. Thus in 1910 only 53.8 per cent. of the population were native whites whose parents had been born in this country, though from 1820 the total immigration had been less than 28 million.

With this chronicle of the past as a basis, one may visualize the future, provided the causes of increase remain the same.

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[10] All U. S. population figures from publications of the U. S. Bureau Census. Quoted figures on other countries from Reports of the Registrar General of England.

In 1891 Pritchett[11] found that the population curve of the United States could be represented by a parabola with the equation $P = A + Bt + Ct^2 + Dt^3 + \cdots$, where P represents the population and t the time from an assumed epoch. How well the projection of this curve has fitted for a short period is shown by the actual and the computed figures for the last three census years. In 1890 they were almost identical; in 1900 there was an excess of nearly a million and a half, and in 1910 an excess of over two million and a half in favor of the computation.

This is a rather good fit, all things considered; but, the fact that the actual population is already lagging behind the predicted population shows that the causes operating in the earlier history of the country have now been modified. The computations of 386 million for the year 2000 and 1,113 million for the year 2100, therefore, are much too high. Nevertheless, these are short periods, 80 and 180 years, respectively, and the tendency toward an almost unbelievable increase is strikingly shown. Let us keep in mind the *possibility* of taking care of 300 million people before all of our children have ceased their struggle for existence, and of supporting some 700 million who will compete with our grandchildren, while we turn our attention to the resources of the country as they exist to-day.

I know I shall be termed a preacher of calamities, but the facts admit of but one conclusion: the law of diminishing returns is even now in operation in this comparatively new country thought to be supplied with inexhaustible riches. This is the result of my own somewhat extensive investigation. It is the result of the accurate scholarly study of Thompson,[12]—the only economist, as far as I have been able to discover, who has had the courage to face the subject in a thoroughly scientific manner.

This age has been called the Age of Steel, an apt trade name for our present type of civilization. Nevertheless this period, like all ages past, and all times to come, is one of Agriculture. Civilization, like an army, marches on its stomach. The present and potential food supply is what interests us most. It is true we could show that in spite of the abundant supply of mineral wealth within our boundaries, all is not as well as might be.

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[11] Pritchett, H. S., "A Formula for Predicting the Population of the United States," Amer. Stat. Assn. Quar. Pub., N. S. 14, Vol. 2: 278–286, 1891. This formula may not be the most precise one possible, but it serves our present purpose.

[12] Thompson, W. S., "Population: A Study in Malthusianism." Privately printed, Ann Arbor, 1915, pp. 216.

Our coal supply is being dissipated. Nearly a sixth[13] of our visible supply of anthracite has been mined within the limits of one generation. But 15,000 tons per capita of bituminous coal still remain untouched,—presumably enough for about 5 centuries with the present per capita consumption. Our petroleum reserve is speeding on its way at a still greater pace. Forty per cent. of the visible supply is gone, and merely at the present rate of consumption there is only enough left for some twenty-odd years.

If, as our mining experts claim, the total supply of these, our two main sources of energy, has been mapped properly, it would seem that our Steel Age is on the road to senility. To a Babylonian returned to look over the prospects, it would probably appear as a transient phase unworthy of particular notice. Perhaps matters are not so bad as they are painted, however. Our utilization of water power is still very limited, and in the more distant future we can look to the moon for our energy requirements. We shall harness the tides. In the case of agriculture, I am not so sanguine.

The sower, the tiller, the reaper, go their ways with the same changeless routine they have taken since the dawn of civilization. No fairy godmother has appeared to aid our most essential art; and the story of the past gives us reason to be skeptical of the future. In a way agriculture has become more efficient. Chemistry, physics and biology in their applied branches have given us a more definite knowledge of cause and effect, and have provided for a greater return per unit of man power. But he who makes two blades of grass grow where but one grew before must be prepared to pay the price in a lesser number of blades to come after. Novel methods of culture, more efficient machinery, new and better yielding varieties, are but means of exploiting a limited reserve of soil fertility at a higher rate.

It is curious what false ideas in regard to agricultural possibilities are held by so many people. Not long ago I asked a well-trained business man how much more land could be brought into cultivation. His off-hand estimate was between 400 and 600 per cent. Now the whole land area of the Untied States is only 1,903 million acres;[14] and 47 per cent. is now included in farms, although only about 55 per cent. of the area of these farms is improved. There remains 1,023 million acres, as a reserve supply. Of this, 468 million acres is arid land having an annual precipitation of less than 15 inches, 200 million acres must be preserved as forest lands, 75 million acres is swamp with perhaps 40 million acres permanently unusable, and 41 million acres is already in cities, roads and railroads. There is then a grand total of 749 million acres which must be withheld from agricultural use permanently, without allowing for urban and transportation increase. When this is subtracted, the 274 million acres left does not loom so large. There is a parcel of 40 million acres to be added, however, when all irrigation projects possible under the present system of construction are made available, making a total of 314 million acres. This calculation shows that we have been extravagant in our optimism. After the expenditure of vast sums, after the completion of tremendous tasks of engineering, we can add barely 35 per cent. to our present farm area.

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[13] Gilbert, C. G. and Pogue, J. E., "The Energy Resources of the United States: a Field for Reconstruction," U. S. Nat. Mus. Bull., 102: 1–165, 1919.

[14] *Cf.* Thompson, W. S., *l. c.*

We may catch at the straw in sight, and point out that only 55 per cent. of farm lands are improved, and that therefore when all available land is improved, 170 per cent. of our present area will be added. But this straw is loose, as a little consideration shows. The unimproved land of to-day remains uncultivated because the net returns for farm produce are not high enough to warrant its cultivation. Bluntly, it is too poor to be farmed until the price of food goes higher. Not only is this true, but it should also be emphasized that many farms in the older parts of our country, which once produced a profit, have so deteriorated after a lone century of cultivation that they are now either tilled at a loss or are abandoned.

It would be foolish to maintain that this country can not support a much larger population. This is not the economic question before us. The population will continue to increase for years to come, though more and more slowly as discomfort and want become more prevalent. My point is that the reserve farm land is less productive than the improved land, that the fertility of the soils now being tilled is decreasing, that the law of diminishing returns is now in active operation. If these are the facts, and the statements are amply supported, a continuous increase in the food cost of living must go hand-in-hand with an increase in population, although there may be a temporary after-war downward trend if production is sustained and the water forced out of our inflated currency. There will be more people, but there will be a more strenuous struggle for existence proportionate to the increase.

What is the reason for asserting that diminishing returns now show in agriculture? There are various ways of throwing light on the question, but data from two sources are sufficient. Elaborate chemical, physical and plot culture investigations of various soils[15] have been made by the Agricultural Experiment Stations of Illinois, Ohio, Pennsylvania and Minnesota, and less extensive experiments have been carried on in other states. One who takes the trouble to study the published reports of these researches with care is fully repaid. They prove beyond question that the farm soils of the United States are by no means of unlimited fertility. The three essential elements of fertility, so-called, potassium, phosphorus and nitrogen are present in such small quantities that the natural production curve of various types of soil under particular systems of cropping can be estimated with a fair degree of accuracy. And in most cases it is not centuries but decades before continuous cropping shows decreasing returns, no matter what the rotation.

Fortunately there is a comparatively large supply of potash in most soils, and there is nitrogen to be obtained from the air by the proper use of legumes or by electrical methods; but the phosphorus content, generally speaking, is so low that it soon becomes deficient if not replaced by artificial fertilizers, for nearly 4,000 million pounds of phosphoric acid[16] are removed annually by the grain crops alone,—nearly 20 pounds per acre. Fortunately again, the element commonly limiting the fertility of the soil may be supplied for a time from the large phosphate beds[17] of South Carolina, Florida, Tennessee and the Northwest, which contain over half of the world's supply, estimated by Hopkins at 500 million tons. The matter of prime importance, however, is not the *possibility* of keeping up crop returns for a considerable period when all available means are utilized. It is, that natural cropping by the present system is depleting all soils rapidly, and that millions of acres have already reached the point where their present productiveness can only be kept up by increasing amounts of artificial fertilizers. Much of our natural agricultural wealth has been used, with no charge made for it in the production costs. This is bad bookkeeping. No charge for depreciation means bankruptcy in any business.

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[15] Hopkins, C. G., "Soil Fertility and Permanent Agriculture," Boston, Ginn, 1910, pp. 653.

[16] Hopkins, C. G., *l. c.*

[17] *Cf.* Wyatt, Francis, "The Phosphates of America." New York, Sci. Pub. Co., 1892, 4th ed., pp. 187.

Now that the fertilizer industry is increasing by leaps and bounds, and farming with the use of artificial manures must compete with new land farming, we can see more clearly what the ledger sheet of the future must show. Absolute costs per unit of man power, and per monetary unit, are mounting and will continue to mount, because of the diminishing returns which such a system entails. Furthermore, there will come a time, a time not so very distant, when the current agricultural system must be replaced. A more efficient system of saving plant nutrients similar to that of China and certain European countries must be installed. The elaborate scheme of waste evolved by our sanitary engineers will have to be discarded.

Perhaps there are those, however, who deny the truth of the current theories of soil fertility. Such denials have been made, though without the support of quantitative data. Nevertheless, be the theories of soil fertility what they may, the experiments on continuous cropping are facts. There is a decrease in *natural* productiveness with all known methods of continuous cropping, which is so rapid it may well alarm those who look toward the future.

Let us examine data on this subject from a second source. They are perhaps still more convincing. We see continually in the various reports of government agencies, how much the crops of the country for a definite year exceed those of a previous year or term of years in acreage, production, and value; yet it is of no particular importance that our production of crops or of food-animals is increasing. Paper wealth is cold comfort. What we wish to know is whether with improved machinery, new methods, and better varieties, our agriculture is keeping pace with our population.

The per cent. of actual increase in population in the decade 1900 to 1910 was 21. The per cent. of increase in farm lands during the same period was 4.8. But this does not necessarily mean a great deal. To obtain more pertinent data I have plotted the *per capita* production of meat animals, and of acreages and production of the chief agricultural crops from 1870 to 1916.[18] In order to obtain trustworthy figures, the enumerations of animals for the census years only have been used, while the production figures are the ten-year averages centering on the census years. The last point is the average for the years 1915, 1916 and 1917. The graphs are very interesting and illuminating, but this is not the place to consider them in detail. The gross results are as follows. The peak of swine production, .951, was reached in 1880. In 1910 it was .633, and in 1916, .667. After various fluctuations sheep production reached .809 in 1900, but in 1916 was only .480. Milch cows have remained rather more constant, but the curve shows a downward trend since 1890. Other beef cattle, perhaps the chief meat supply, have dropped markedly from a high point of .666 in 1900 to .399 in 1916.

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[18] The figures upon which these calculations are based, have been taken from the Year books of the U. S. Dept. of Agriculture.

The vast grazing lands of the early days are gone and with them low priced meat has passed. Does one need any other reason for the rise in meat price-rates up to the year 1915?

Let us pass now to the per capita acreage of the great cereals and potatoes. Corn remained the same from 1880 to 1890, about 1.16, and has dropped slowly ever since. Wheat reached a peak of .67 in 1880, and has steadily gone down to .52. Oats was the same in 1916 as in 1890, but was lower *ad interim.* Rye and potatoes have remained rather constant, though the tendency is downward. The barley acreage has risen through the demands of the liquor interests, but may be expected to drop with the incoming of prohibition. Thus in the pre-war period from 1870, the acreage of three great cereals, corn, wheat and oats, is not a matter of congratulation, though production through the increased use of fertilizers, better varieties and more intense cultivation has been sustained.

The production history for corn in the decades from 1870 taking for the last point the average of the years 1915, 1916 and 1917, is 23.38, 29.43, 27.28, 27.90, 29.72, and 28.61 bushels. As a whole, then, corn production has just about kept pace with population. Wheat, the basis of national life, follows nearly the same course. The figures 6.01, 8.24, 7.24, 7.60, 7.23 and 7.59. Oats have risen sharply. The figures are 6.63, 8.65, 10.41, 10.49, 11.17, 14.39.

These figures, considering them from all angles, are not a cause for present pessimism. The increased production of 1918 and 1919, due to the stimulation of war prices, shows what may be done by intensive methods even with our present system of agriculture. Nevertheless, I ask you to note that as a whole our food production per capita from about 1880 or 1890 to the war period has slighly declined. And this is in face of a tremendous increase in the use of fertilizers, a widespread propaganda for better methods of cultivation which was not without its results, a constant influx of improved machinery, and a continuous production of new and better yielding varieties. There is but one conclusion. Diminishing returns in agriculture *are operating*, and with a continuously and rapidly increasing population they will become more and more in evidence.

The arguments that have been used concerning diminishing returns in agriculture are not negated by the fact that the United States is still exporting large quantities of food. Since the people of the United States do not at present eat all the food produced, many will say that all is well. This does not follow. The improvident individual may feast before he wants. The prodigal heir may have a high time before the reckoning comes. The business on the verge of bankruptcy may be running at the peak of production. No one denies that the productivity of the United States is at present beyond her needs. No one denies that this production may be increased very considerably. We do maintain that capital is being dissipated to show these results, that returns are lessening to such an extent that the food cost of living—barring the war disturbance—is bound to rise, and that certain portions of the country actually do feel the pressure.

It may be replied that decreasing returns in agriculture will be offset by increasing returns in non-agricultural industry. But, as Thompson has shown, it is not at all certain that manufacture presents increasing returns. It is but recently that cost accounting has taken into consideration the case of the workman, and it has not yet included the cost of maintaining our great city industrial systems with their streets, bridges and railways, their schools and hospitals, their banks and libraries, their bonded indebtedness. With these increased charges, what would be the balance sheet? The very fact of the immense bond flotations of our political units show that we are building upon the insecure assumption that the present social system is sound and permanent. Unless increasing returns for the country as a whole are in sight, our house is built upon sands. Let us give these matters their proper place when dealing with panaceas for social unrest. There can be no value in prescriptions which leave untouched the fundamental causes.

If pressure upon the means of subsistence is beginning to be felt in this relatively new country, if misery and suffering due to overcrowding is approaching—and who can face the facts and continue to doubt—what a dire prospect exists for the older countries of the world.[19] And a sad feature of the case is the fact that though there be a continuous emigration to those portions of the world where unexploited natural wealth still remains, but slight temporary relief will be afforded the countries from which the emigrants go. *Long before the sparsely populated lands are filled, in the next half century in such prosperous countries as our own, there must be a shift of population from the cities to the farms.* Coincident with this industrial change there must be a simplification and probably a lowering in what we are pleased to call the standard of living. Technical industry can not take the burden of this shift and still continue to furnish the whole of the people with the comforts and luxuries they have come to deem necessary. Contemplation of this fact is all that is necessary to show that at the same time there will be a continued lowering of the birth rate. The relation between these matters is not kept from the people. Their theories on the subject may be hazy, but direct pressure on existence is a great teacher. The birth rate will continue to go down, and immigration will be greatly restricted if not prohibited. Broadly speaking, this is a result not to be deplored. Why then is so much time, ink and paper used in decrying the declining birth rate? There is one real reason, but it is not the one acknowledged by greater population propagandists.

Economically the position of the French people in 1914, with a very slowly increasing population, was extraordinarily good. Thrift and industry had placed them in the fore-front of the nations of the world, and there was relatively little pressure upon the means of subsistence. They feared two things: first, immigration of peoples with lower standards of living and greater rates of natural increase, with resulting replacement of the native French; second, attack from the east. Both fears had a real basis as we know, and Leagues of Nations or of

[19] One of my colleagues has made the off-hand criticism that these conclusions do not hold because no allowance has been made for the "immense possibilities in the way of utilizing sea food." This criticism well illustrates the ease of refuting a statistical argument by using a statement which has no basis of fact. In the first place, the sea-food industry of the world is of little import as a factor in feeding the world's population. Making all due allowance for possible understatement in the statistical returns, the value of the sea-food production of the world is *less* than that of the production of poultry and eggs in the United States. In the second place, diminishing returns in this industry are indicated by the fact that the increase in capital employed during the last 20 years is very much greater than the increase of the returns in product.

Notions will not abate them. These apprehensions will always be bogies of small nations. The odd thing is that so many statesmen should encourage actual misery from over population to alleviate a fear of possible misery through aggression.

In a country such as the United States the second fear is groundless. With a proper preparation for war, an attack on the United States should not be considered seriously. Mere masses of people are not a menace as they once were, as the Germans and Russians have demonstrated. And we are big enough to take care of ourselves. The other fear is something more definite, but few give it any attention. The unacknowledged reason why the politician wants more people is to satisfy his longing for greater power, the hidden desire of too many capitalists is for cheap labor. The real objection to a declining birth rate is that it is always selective.

The selective birth rate in the United States is a subject on which much has been written, but on which there is little accurate information. The vital statistics of various states are meagre and rather untrustworthy. In fact even at the last report (1916),[20] the registration area included only about one third of the whole population. The crude birth rate for that year was reported as 24.8 and the crude death rate 14.7, leaving a natural increase of 10.1 per thousand; but the figures probably do not represent the facts. Neither death rates nor birth rates are reported in full, but the deficiency in registration of births is undoubtedly greater than that of deaths. It would not be a surprising matter if the real natural increase in the United States as a whole is about 11.5.

The negro birth rate[21] is put at 22.8 and the death rate at 24.4. Obviously these figures can not be accepted at their face value. Not only are the vital statistics registered for negroes known to be less accurate than those for whites; but no part of the real "black belt," where the negro is in his best environment, lies within the registration area. The figures are largely from cities where there is an extensive negro population, places in which numerous factors tend to lower the natural increase from that attained in the "black belt" even though the economic condition based on per capita wealth may seem higher. The census figures for 1910 give some idea of the discrepancy.

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[20] Birth statistics for the registration area of the United States (1916). Second Annual Report. U. S. Bur. Census, 1918, pp. 96.

[21] The Negro Population in the United States, 1790–1915. U. S. Bur. Census, 1918, pp. 844.

In the previous decade the negroes increased 993,769, 11.2 per cent., and this may be taken as largely from natural increase, since there was an increase of only 20,003 foreign born, and this figure must be diminished by about 45 per cent. to allow for emigration. During the same period the whites increased by 22.3 per cent. The proportion of negroes in the population as a whole is decreasing, therefore, yet one can not help but feel that if all the data were at hand, the natural increase of the whites and the negroes would be found to be about the same. If venereal diseases are ever stamped out among them, and a reasonable degree of sanitation instituted, there is scarcely a doubt but that they will tend to supplant the whites, for the whites have kept their numerical superiority largely through immigration and a better knowledge of hygiene. Thus, since there were nearly 10 million negroes in continental United States in 1910, the color problem is not the negligible matter some of our northern sociologists would have us think.

Another fact which should be regarded seriously is that the mixed bloods are increasing much more rapidly than the pure blacks. There were nearly twice as many mixed bloods in 1910 as in 1890. In 1890 the mulattoes formed 15.2 per cent. of the negro population, in 1910 they represented 20.9 per cent. With the white blood comes greater intelligence, a lower death rate, and in all probability a higher birth rate. In time segregation and recombination of traits will result in a considerable number of people with negro blood who will pass for white.

It seems hardly necessary to point out the undesirability of this situation. The negro is a happy-go-lucky child, naturally expansive under simple conditions; oppressed by the restrictions of civilization, and unable to assume the white man's burdens. He accepts his limitations; indeed, he is rather glad to have them. Only when there is white blood in his veins does he cry out against the supposed injustice of his condition. White germplasm in a negro complex spurns its hopeless situation, as Humphrey[22] notes. Yet the result of such a wide racial intermixture is a mediocrity; which, owing to the numerous gametic differences linked and coupled in an infinite complexity of ways, will tend to remain a mediocrity till the end of time.

Concerning the potentialities of our white "Melting Pot," one is likely to say too much or too little. As a mere matter of statistics the country seems to be progressing fairly well. There is a somewhat greater proportion of foreign than of native paupers, but that might be expected without great discredit to the former. In 1910 there were .88 per thousand native and 1.57 per thousand foreign-born prisoners and juvenile delinquents,[23] but in all probability the sins of the good American citizen did not find him out as often as might be desired. At the same period there were 1.69 per thousand natives and 4.05 per thousand foreigners in institutions for the insane. No doubt the terrific whirl of American life drove some of these newcomers mad, for the proportion is rather different in institutions for the feebleminded. The ratio there per thousand is, native 2.65, native of native parentage 1.70, native of foreign parentage 2.86, and foreign born, .93.[24]

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[22] Humphrey, S. K., "Mankind," New York, Scribner, 1917, pp. 223.

In acquiring some slight knowledge of letters the foreigners coming to our shores have done well. In our native white population born of native parents 3.7 per cent. were unable to read and write in 1910, but although 12.7 per cent. of the foreign born were in the same predicament, hardly one tenth as many natives born of foreign or of mixed foreign and native parents are to be so classified.[25]

In spite of this somewhat unexpected showing from our recent emigrants, however, there are reasons for serious misgivings, misgivings sufficient to warrant us advocating such restrictions as to practically prohibit immigration for a period of time that will at least permit us to consider the subject in all its aspects. The examinations in the selective draft, the radical labor troubles now in full fruit, the Bolshevist propaganda, have shown how refractory are some of the ores in the melting pot and how poorly our fires have been tended.

We have developed too rapidly, and are experiencing acute growing pains in various portions of the anatomy of our body politic. It is troublesome and should be attended to, but it is not extremely serious. If we continue as in the past, we shall suffer indeed. We must come to realize that if we make the most of our grand heritage of democratic ideals left by the fathers of colonial days, we must change our tactics.

The point, it seems to me, is this. Our political legacy, our folkways, our Americanism, is North European, Northern Aryan, Nordic. Whatever one wishes to call it, its origin is not in doubt. Our great men in all lines—statesmen, warriors, writers, scientists, inventors—came so largely from this ethnic mixture that if they are excluded but little is left. Some one once said: Take from France her hundred great ones, and where is France? We may paraphrase it thus: Take from Columbia her Anglo-Saxon sons, she is bereaved indeed. And is she not being dispossessed of her Anglo-Saxon stock? The birth rate of our foreign population, coming so largely now from eastern and southern Europe, is so much greater than that of the Anglo-Saxons that within a century the latter will be but a fraction of the whole.

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[23] Prisoners and juvenile delinquents, 1910. U. S. Bur. Census, Bull. 121, 1914, pp. 130.

[24] Insane and feeble-minded in institutions. U. S. Bur. Census, 1914, pp. 217.

[25] Illiteracy in the United States. U. S. Bur. Census, Bull. 26, 1905, pp. 54. Also 13th Census of U. S.

It may be that the various ethnic mixtures recently acquired will do their full share toward contributing to the progress of civilization. There are many, however, who feel justified in maintaining that if immigration had been restricted in the middle of the nineteenth century, the country would be better off physically, morally, mentally and economically. There would be almost as many people within its limits, for the superior types of the early days would not have been forced to the wall by the avalanche of progeny begotten by the horde of aliens. Given the time these matters may right themselves, but it should not be forgotten that it is just such waves of immigration that overturn civilizations before there is time for readjustment.

We are entering such a period. We must fight for time, or accept an overturn of the present social order. No doubt it is desirable to have social changes; it is suicidal to allow a sudden economic chaos. Among biologists a defense of private property, free enterprise, and a competition which does not interfere with social order, is unnecessary. These things must be, in order to bring out the fittest to survive. At the same time there must be a regulation of the degree of social warfare or a reversion to savagery will result. People are much alike. The capitalist takes everything when he has the chance; the workman hopes to take everything when he is able. Obviously this natural law promotes progress; but in society even as in nature, the most rapid progress comes when the selective action is directed along certain channels and restricted to particular ends. The great effort, then, must be to direct it properly. Denying its existence is of little avail.

What is the answer? As I see it, it is this: First a severe restriction of immigration; second, education; third, equitable readjustment in many of our economic customs; last, but by no means least, rational marriage selection, a somewhat increased birth rate in families of high civic value, and among the rank and file a restriction of births commensurate with the family resources and the mother's strength.

Economically we can not afford a high birth rate, but it should be cut in the proper place. If this be done, it means a fall in the death rate, in the disease rate, in the proportion of misery and poverty. It means less child labor and illiteracy, less prostitution and venereal diseases. It means a healthy home life, larger production, greater economical freedom and therefore happiness. Anything else is merely reducing the fit to the level of the unfit.

If we have the brains, the energy and the courage to put through such a program there is some hope of escaping the whirlpool into which we are drifting. The ingredients in the melting pot are not all bad by any means, and—one may be thankful for it—they do not form an amalgam. Each and every ethnic contribution carries some hereditary factors making for a good or even a great individual. The result of the interbreeding of the next century will on the whole be mediocrities, as it has been in the past, but recombination will give here and there the individual with the characters that make for genius and progress will be assured. If we do not open our eyes on this problem of population, however, there will be troubles in the future which will make those of the present seem like the tempests of a teapot.

GENETIC STUDIES ON THE PROTEIN CONTENT OF MAIZE

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Reprinted from GENETICS **5**: 543–610, November, 1920

GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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All subscriptions, notices of change of address, and business correspondence should be sent to the WILLIAMS & WILKINS COMPANY, Baltimore, U. S. A., and all remittances should be made payable to the WILLIAMS & WILKINS COMPANY.

Entered as second-class matter February 23, 1916 at the post office at Princeton, N. J., under the act of March 3, 1879. Application for transfer of the publication to Baltimore, U. S. A., has been requested.

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[Received May 4 1920]

TABLE OF CONTENTS

INTRODUCTION 543
Results of previous work 545
The inheritance of protein in maize 554
  The problem 554
  Variation in the protein content of individual seeds 557
  Comparison of the protein content of self-pollinated and of wind-pollinated ears 562
  The immediate effect of pollination on the size and composition of the seed 563
  The protein content of different ears borne on the same plant 573
  The immediate effect of pollination on different ears borne on the same plant 573
  The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants of the same strain 576
  The protein content of first-hybrid-generation plants bearing second-hybrid-generation seeds 578
  Conclusions regarding the inheritance of protein in maize 582
Experiments on breeding for high protein 589
  Original experiments on selection 589
  Selections in self-fertilized lines 590
    Stadtmueller's Leaming 590
    Illinois High Protein 595
    Illinois Low Protein 596
    Burwell's Flint 597
  Conclusions regarding selection for high protein in self-fertilized lines 598
  Selected matings between high-protein plants 600
  Conclusions regarding breeding for high protein 608
LITERATURE CITED 609

INTRODUCTION

Agricultural crops owe their popularity to a variety of qualities. If they will keep for long periods, if they can be stored economically and shipped easily, if they suit all palates, their usefulness is naturally enhanced. But, other things being equal, their content of particular desirable constituents determines not only their true worth, but their commercial value. Such a statement is no more than a platitude when it concerns plants grown for fibers, drugs, dyes, tannins, rubbers, or essential oils. In the great food plants, the matter is not so obvious; yet data collected some months ago by one of us, indicate a rather striking class difference and noteworthy uniformity within each class in the prices people have come to pay for carbohydrates, fats, proteids and vitamine carriers, when correction is made for the variables noted above.

Naturally, appreciation of differential values in plant constituents has led to many plant-breeding projects in which the chief aim has been to develop the desirable quality rather than to increase the yield of the crop. One cannot criticize efforts to raise the quantity and quality of fiber in cotton and flax, or the yield of rare oils and drugs. In other cases one cannot be so certain that such efforts are advisable. It may be true, for example, as maintained by HOPKINS and SMITH, that there is demand for maize varieties rich in high or low protein or in high or low oil. It is possible, however, that the best economic practice is to grow other plants for these particular purposes. If the difficulties in the way of breeding wheat and maize for high protein content or maize for high oil content are extreme, it is probably wiser to obtain proteid concentrates or commercial oils from plants naturally rich in these substances. Nevertheless facts regarding the inheritance of such chemical constituents as protein are always valuable, and the writers submit this paper hoping that the results set forth have some present value to genetic theory and the possibility of future value to agricultural practice.

The work is based upon an experience of nearly twenty years. From 1900 to 1905, familiarity with the problems involved was gained by contact with the maize-breeding projects of the ILLINOIS AGRICULTURAL EXPERIMENT STATION. In 1906 mass-selection experiments were started at the CONNECTICUT AGRICULTURAL EXPERIMENT STATION. It was soon apparent, however, that information additional to that secured by the ILLINOIS AGRICULTURAL EXPERIMENT STATION was not likely to be obtained until more was known about the inheritance of simpler characters. Accordingly the investigations on protein inheritance were held in abeyance for several years. Since 1909, however, the work has been prosecuted with some vigor by Professor H. K. HAYES now of the UNIVERSITY OF MINNESOTA and by the authors, as a coöperative project between the CONNECTICUT AGRICULTURAL EXPERIMENT STATION and HARVARD UNIVERSITY. The funds for the plat work were furnished wholly by the first-named institution, the pedigree cultures were grown upon its experimental farm, and the chemical determinations were made within its laboratories,—in earlier years by the authors, in later years by the staff of the Chemical laboratory. Current analyses of the results and plans for each year from 1909 to 1917, were made first by H. K. HAYES and E. M. EAST and later by D. F. JONES and E. M. EAST during the winters in the laboratories of the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

RESULTS OF PREVIOUS WORK

It would serve no useful purpose to describe in detail the early plant-breeding work wherein the chief object was to enhance the value of a plant by increasing the production of a particular chemical constituent. The classical example, and one of the earliest projects for breeding plants on a large scale, is the work of the French chemists and agriculturists on the sugar beet, inaugurated in the early part of the nineteenth century by the great Corsican, and carried on continuously since that time both in France and in Germany. Similar work on the sugar cane has been carried on in Java and to a limited extent in Cuba. No other comparable work of such magnitude or of such commercial importance exists, yet each year sees the initiation of some plan of this kind on a small scale. There are schemes for increasing and decreasing protein in wheat and maize, for increasing oil in maize, peanuts, castor beans and soy beans, for obtaining greater yields of the essential oils used in perfumes and of the valuable ingredients in certain drug plants. And recently both English and American companies have undertaken work designed to augment the yield of rubber latex in *Hevea brasiliensis*.

Few of these projects have resulted in any supposed or actual increase in genetic knowledge. In nearly every case mass selection has been practised with no more powerful tool of knowledge than the empiric formula "like tends to produce like." Even the numerous German contributions to literature on the sugar beet have had no effect on current genetic thought. They have resulted in a better knowledge of the varying composition of different parts of the root and in the effects of factors of environment on the elaboration of sugar, but even today one cannot say with certainty whether new variations have occurred which aid in the production of higher sugar content. Elimination of lines with a low sugar content, and accumulation of favorable genetic factors by segregation and recombination may account for everything in the sugar beet, though one hears much about the *change* in the ability of the plant to produce sugar. It may be doubted whether the extreme individual beet is higher in sugar today than in the year 1800, but there are more of these high individuals.

An exception to these remarks is the work carried on since 1896 at the ILLINOIS AGRICULTURAL EXPERIMENT STATION, where the primary object has been to establish commercial strains of maize characterized by high or low protein content, or by high or low oil content, but where the idea of contributing to genetic knowledge has never been allowed to lapse. It is natural, therefore, that these experiments should be described in some detail in this paper.

In 1892, JENKINS and WINTON compiled the published analyses of American feeding stuffs. Among them were analyses of maize seeds produced by plants of different varieties grown in various parts of the country. The range and average of protein content calculated to water-free material are shown in table 1. These reports of protein are in reality determinations of total nitrogen multiplied by the factor 6.25. That this factor is sufficiently correct for all practical purposes is demonstrated by the work of CHITTENDEN and OSBORNE (1892) where the weighted average percent of nitrogen in the different proteid bodies was found to be 16.00.

TABLE 1

Percentage protein content of maize seeds in water-free material.

Type	Number of samples	Maximum	Minimum	Average
Dent	86	13.8	8.2	11.5
Flint	68	14.0	7.7	11.8
Sweet	27	17.0	10.3	12.8
Pop	6	14.4	11.0	12.5
Soft starch	5	15.5	9.5	12.5

There are various proteids in maize as these investigations show:

	Percent
1. Proteose soluble in water	0.06
2. Very soluble globulin	0.04
3. Maysin soluble in dilute salt solution	0.25
4. Edestin soluble in concentrated salt solution	0.10
5. Zein soluble in alcohol	5.00
6. Proteids soluble in dilute alkalies	3.15
7. Proteids insoluble in these solvents	1.05

Thus zein is the important proteid, comprising over 50 percent of the total. Unfortunately zein lacks the essential amino acids, glycocoll, lysine and tryptophane, and contains relatively small amounts of arginine and histidine; hence it cannot be used as the sole proteid food in building up animal tissues, as has been shown by OSBORNE and his co-workers in numerous investigations.

Variations comparable to those of table 1 were found in all the constituents of maize, and gave HOPKINS the basis for starting the ILLINOIS AGRICULTURAL STATION experiment. Admittedly, a considerable portion of these deviations were due to varying environmental conditions, but he was satisfied that there were heritable differences in composition which could be made the basis of selection. He was not able to prove this at the time, but offered some presumptive evidence in the shape of individual analyses of 50 selected ears of Burr's White maize grown on a field having particularly uniform soil conditions. The frequency distribution of proteins and of oil contents (table 2), showing, as they did, deviations as great as the compiled analyses of maize grown under extremely varied conditions, were accepted as indicating differences due to heredity.

TABLE 2

Frequency distribution of percents of protein and of oil in 50 ears of Burr's White maize grown under uniform soil conditions.

Classes	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0
Distribution of protein	2	3	2	6	8	8	6	9	2	3	0	1

Classes			4.0	4.3	4.6	4.9	5.2	5.5	5.8	6.1		
Distribution of oil			5	9	11	16	5	3	0	1		

HOPKINS assumed, with little biological, but with considerable pragmatical justification, that the ear of the maize plant may be taken as a unit. Marked variations were found when samples of seed were taken from top, middle or butt seeds, but several samples of three rows of seeds taken from the whole length of the ear showed very small deviations (table 3). Similarly the comparative uniformity of the protein content of single seeds from the same ear—after 3 years of selection for protein—was later taken as warranting the conclusion that the composition of the ear is approximately uniform throughout (table 4).

Starting with these assumptions, 163 ears of Burr's White maize were analyzed (HOPKINS 1899). Considering only the protein and the oil contents, the distribution of the analyses was as follows (table 5). From selected ears of this lot, breeding in four directions was begun,—(1) high protein, (2) low protein, (3) high oil, (4) low oil. In general the method was to select twenty-four ears of each type by chemical analysis every year, and to continue each line in an isolated breeding-plot by the ear-row method. The main criterion of selection was the chemical composition, but naturally some attention was paid to the appearance of the ear and to its yield as measured by the resulting progeny.

TABLE 3

Deviation in percentage of protein in random samples from single ears.

Ear number	Sample from		
	Tip	Middle	Butt
1	11.77	12.24	12.39
2	11.98	12.49	13.06
3	9.70	10.08	10.48
4	10.60	11.04	11.00
5	10.82	11.33	11.30
	1st 3 rows	2nd 3 rows	3rd 3 rows
1	10.77	10.96	10.69
2	11.99	12.03	12.14
3	10.10	10.16	10.18
4	10.46	10.26	10.08
5	11.20	10.64	10.86

TABLE 4

The percentage protein content of individual seeds on the same ear.

Seed number	Ear number				
	1	2	3	4	5
1	12.46	12.17	11.53	7.45	7.72
2	12.54	12.94	12.32	7.54	8.41
3	12.44	12.51	12.19	7.69	8.37
4	12.50	13.42	12.54	7.47	8.31
5	12.30	13.12	12.14	7.74	8.02
6	12.49	14.49	12.95	8.70	8.76
7	12.50	13.21	12.84	8.46	8.89
8	12.14	13.43	12.04	8.69	9.02
9	12.14	13.16	12.75	8.86	8.96
10	12.71	14.05	—	8.10	8.89

The desire to keep up the yield and to preserve a good physical type, as well as the fact that selection was made only through the mother, obviously prevented a rapid shift of type; yet the results were rather remarkable. Through the kindness of Dr. L. H. SMITH we are enabled to quote the gross averages up to the year 1919.

In table 6, the crop averages for the high-protein and the low-protein plots are presented. There is an increasing difference in the protein content of the two strains which continues until the high-protein type contains 8.17 percent more protein than the low-protein type. When the data are shown graphically (figure 1), two facts stand out impressively. The shift in the average is rapid at first; but becomes slower and slower, until the shape of the fitted curves are changed from concave to convex at about the sixth or seventh generation. Nevertheless there is a continuous shift of the average, and apparently the end is not in sight. Second, the influence of environment is very marked. The protein content of the two plots goes up or down according to the season, but always the difference between the two increases.

TABLE 5

Distribution of protein and oil in the 163 ears of maize with which the Illinois Agricultural Experiment Station maize-breeding investigations were started.

Oil, percent	Protein, percent													Total number of ears
	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	
3.8								1						1
4.0			2	2			2	3	1	1	1			12
4.2				3	2	3	3	2	3		2			18
4.4				3	4	2	7	3	2					21
4.6			2	1	1	8	5	8	4	3				32
4.8	1	1		5	6	3	7	2	4	5				34
5.0		1		2	1	3	3	5	4		1			20
5.2				2	3	2	2	1		3	1			14
5.4						2								2
5.6				1	1	1	1		1		1			6
5.8							1						1	2
6.0					1									1
Total number of ears	1	2	4	19	19	24	31	25	19	12	6	0	1	163

Table 7 shows the figures for the high-oil and the low-oil plots in the same way. The high-oil strain in 1918 has finally reached the remarkable figure of 9.35 percent, while the low-oil type contains only 1.87 percent. Graphically the curves (figure 2) for the changes in oil contents show rather more regularity than is the case with the protein. The rate of change is comparatively constant, seasonal differences apparently having little to do with the matter.

The remarkable results obtained in these experiments have been the object of much comment; and, as BABCOCK and CLAUSEN (1918) point out, the theoretical interpretations of the rôle which selection played, have been varied. The conclusions of Hopkins and Smith (1903–1917), of Davenport (1908) and of Castle (1916), have been similar in that they seem to attribute a peculiar creative power to selection which meets with a certain response on the part of the plant. The reason for these conclusions appears to be in part an adherence to the Darwinian idea that all fluctuations are heritable and that continuous selection is therefore always effective in shifting the type; in part a scanty appreciation of the results of other pedigree-culture work; and in part a failure to realize that the unit of selection is the seed and not the ear, combined with a lack of appreciation of the variables which come into play when a system

Table 6

Results of selecting maize for high and for low protein content at the Illinois Agricultural Experiment Station. Average percent protein in crop each generation.

Year	High strain	Average for period	Low strain	Average for period	Difference	Difference for period
1896	10.92		10.92			
1897	11.10		10.55		0.55	
1898	11.05		10.55		0.50	
1899	11.46		9.86		1.60	
1900	12.32	11.37	9.34	10.24	2.98	1.13
1901	14.12		10.04		4.08	
1902	12.34		8.22		4.12	
1903	13.04		8.62		4.42	
1904	15.03		9.27		5.76	
1905	14.72	13.85	8.57	8.94	6.15	4.91
1906	14.26		8.64		5.62	
1907	13.89		7.32		6.57	
1908	13.94		8.96		4.98	
1909	13.41		7.65		5.76	
1910	14.87	14.07	8.25	8.16	6.62	5.91
1911	13.78		7.89		5.89	
1912	14.48		8.15		6.23	
1913	14.83		7.71		7.12	
1914	15.04		7.68		7.36	
1915	14.53	14.53	7.26	7.74	7.27	6.79
1916	15.66		8.68		6.98	
1917	14.44		7.08		7.36	
1918	15.48		7.31		8.17	

of breeding by selection through the mother is practised. DAVENPORT and RIETZ (1907), for example, studying the four strains by statistical methods after ten years of selection, use the ears as units of discussion and conclude that "the variability was not sensibly reduced during the ten years of rigid selection." Their study of the data, led them to believe that "after great improvement has been secured there is still left abundant variability on which to base future selection, and that if the limits of improvement are ever reached it will be for some reason

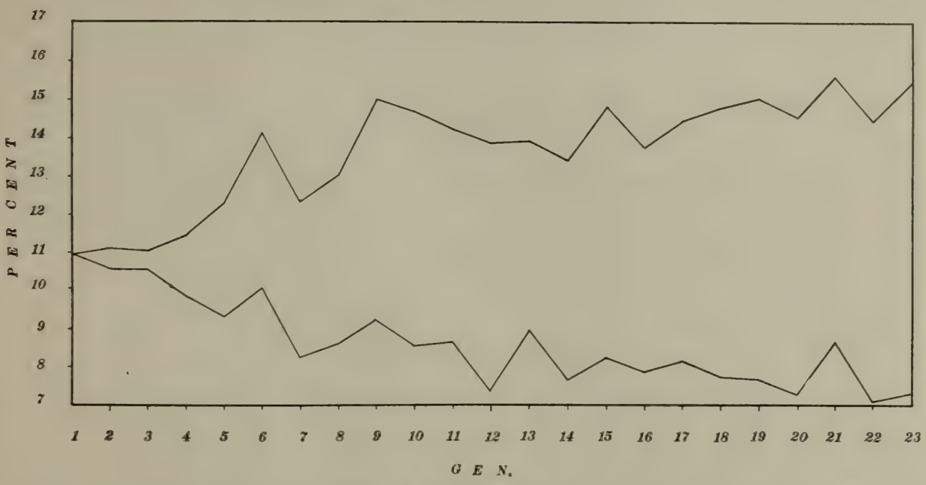

FIGURE 1.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high protein and for low protein.

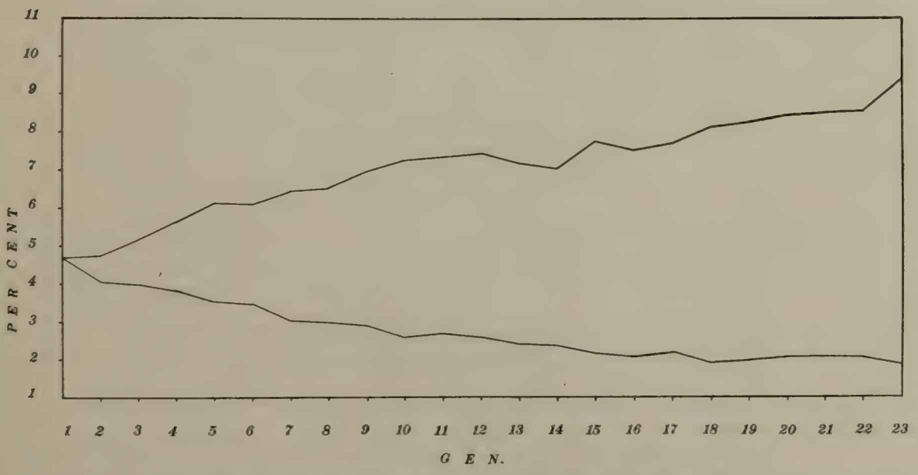

FIGURE 2.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high oil and for low oil.

other than the failure of variability." In other words, "the effect of selection is to shift the type without greatly altering variability."

These data do indeed show that the effect of selection has been to bring about the production of four types as distinct in their physical characteristics as in their chemical composition; but if one realizes the slowness of obtaining particular Mendelian recombinations when indiscriminate pollination is resorted to, and appreciates the statistical result when the ears are regarded as the units of heredity, it is clear that other interpretations are possible. In passing it may be said that the ear is a population of seeds; and it can be shown graphically (figure 3) that selection may take place for many generations, reducing the variability

TABLE 7

Results of selecting maize for high and for low oil content at the Illinois Agricultural Experiment Station. . Average percent oil in crop each generation.

Year	High strain	Average for period	Low strain	Average for period	Difference	Difference for period
1896	4.70		4.70			
1897	4.73		4.06		0.67	
1898	5.15		3.99		1.16	
1899	5.64		3.82		1.82	
1900	6.12	5.41	3.57	3.86	2.55	1.24
1901	6.09		3.43		2.66	
1902	6.41		3.02		3.39	
1903	6.50		2.97		3.53	
1904	6.97		2.89		4.08	
1905	7.29	6.65	2.58	2.98	4.71	3.67
1906	7.37		2.66		4.71	
1907	7.43		2.59		4.84	
1908	7.19		2.39		4.80	
1909	7.05		2.35		4.70	
1910	7.72	7.35	2.11	2.42	5.61	4.93
1911	7.51		2.05		5.46	
1912	7.70		2.17		5.53	
1913	8.15		1.90		6.25	
1914	8.29		1.98		6.31	
1915	8.46	8.02	2.07	2.03	6.39	5.99
1916	8.50		2.08		6.42	
1917	8.53		2.09		6.44	
1918	9.35		1.87		7.48	

of each line-bred family in a marked degree, without necessarily reducing the variability of averages of population samples which are obtained by analyzing ears. It is shown in figure 3, for instance, that a normal frequency surface may be constructed in which the variability in one direction—measuring a series of samples—may remain the same, though the variability of the sub-population making up each sample, is very different.

The interpretation noted above has been given up by CASTLE (1919) because of the steadily increasing evidence that recombinations of Mendelian factors account for the results obtained, in a simpler and more helpful manner.

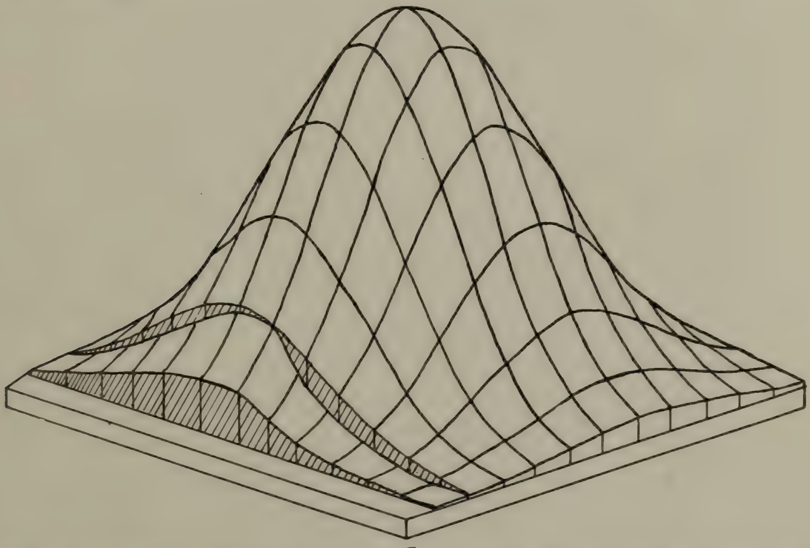

FIGURE 3.—A normal frequency surface showing how the dispersion coefficient may be changed in one direction without affecting it in the other direction.

A Mendelian interpretation of the Illinois results, isolation of various combinations of hereditary factors, was first suggested by EAST (1910), on the general basis that it was more plausible to have an analysis of these facts in keeping with modern genetic interpretations of analogous phenomena, and for the specific reason that the fitted curves showed a retardation in the effects of selection.

This stand was strongly supported by an analysis of the pedigrees of the four Illinois strains after ten years selection, made by SURFACE in 1911. He found that the 24 "High-protein" ears selected for planting in the eleventh generation, all traced back to one original ear, No. 121; of the "Low protein" ears, 20 traced back to No. 106 and 4 to No. 107;

of the "High oil" strain, 12 ears came from No. 111, 4 ears from No. 114 and 8 ears from No. 118; and of the "Low oil" strain 16 ears originated from No. 106 and 8 ears from No. 110. Thus in the eleventh generation all of the 96 ears of the four strains traced back to 8 ears of the original Burr's White, a rather convincing demonstration that the results of selection were mainly the accumulation of hereditary complexes effective in various ways, though of course no one could maintain that mutations had not ensued during this lapse of time.

Other experiments modeled along lines similar to these have been carried on at various agricultural experiment stations since the first report of the Illinois investigations, but so far as we know only one resulted either in new facts or in a new point of attack. We refer to that of PEARL and BARTLETT (1911). In this study a cross was made between a white sugar corn and a yellow dent, and F_2 seeds of the four classes, yellow dent, white dent, yellow sugar and white sugar, were analyzed and the results compared with those obtained from seeds of the parental and F_1 generations. Moisture, nitrogen, ether extract, ash, crude fiber, pentosans, sucrose, dextrose and starch were determined. No discussion of the relative accuracy of these determinations was made, but it was thought that high moisture and high starch dominated the alternative conditions, low moisture and low starch, while in the remaining constituents the lower percentage dominated the higher percentage. Segregation was obvious in every case. It was not shown, and probably the authors would not now maintain, that single factors determined the difference between "high" and "low" content of any of these complexes; moreover, matters other than simple segregation in the usual sense must be taken into consideration in such a genetic analysis, as we shall show; but the authors deserve great credit for bringing out the fact that the seed and not the ear must be taken as the unit in any such study.

THE INHERITANCE OF PROTEIN IN MAIZE

The problem

The genetic problems involved in an effort to change the chemical composition of maize by breeding cannot be understood clearly unless the elementary botanical facts connected with seed formation are borne in mind. This would seem to be, and ought to be, an unnecessary observation; yet a careful survey of the statements made by previous investigators leads one to believe that ignorance or carelessness regarding these facts has led to numerous erroneous conclusions.

One should expect the composition of the maize seed to be influenced both by the genetic constitution of the mother plant and by the environment under which it develops, but it should not be forgotten that the grain itself, speaking botanically, is in a sense a young zygote having characters of its own derived from the gametes from which it is formed. Classified according to their origin, however, there are these distinct parts to the seed,—the pericarp, a maternal tissue, the embryo formed by the union of the egg with the first male nucleus, and the endosperm formed by the union of the second male nucleus with the so-called endosperm nucleus—a fusion product of two embryo-sac nuclei. The line of hereditary transmission is confined to the gametes produced by the plant maturing from the embryo, but the composition of the seed is determined largely by the composition of the endosperm which forms about 80 percent of each individual kernel. Now the cytological and the pedigree-culture evidence are in agreement that the above method of seed formation is so rigid in the species under consideration that no one has been able to establish an exception. These experimental methods have also demonstrated (see EAST 1913), first, that from the chromosome standpoint the embryo is a $2x$ body and the endosperm a $3x$ body; second, that the two "male" nuclei on the one hand and the three "female" nuclei on the other hand, have respectively the same genetic composition. If a male nucleus entering into endosperm formation bears a factor through which a particular character develops, therefore, one may rest assured that the "brother" nucleus entering into the formation of the embryo, will bear the same factor. And the same is true of the three "female" nuclei. Nevertheless some complications arise, due to this double-fertilization process, which make the various genetic phenomena involved somewhat difficult to analyse, although the basis upon which such analysis must depend is quite clear.

For example, in the earlier investigations on inheritance of maize-endosperm characters, such characters as the yellow ether-soluble pigment, the blue and red anthocyans of the aleurone cells, and the presence and absence of starch development, it was found that the endosperm could be considered to be identical with the embryo without error. The dominant characters seemed to show the same degree of dominance, a degree approaching perfection, no matter whether they entered from the male or the female side. In other words, a single nucleus contributing certain factors from the male side, seemed to exert the same influence on development as a double ($2x$) nucleus entering from the female side. HAYES and EAST (1915), however, found that this simple behavior was not characteristic of every character. In the starch differences causing the chief distinction between the floury types and the horny or translucent types, dominance followed the maternal side. The two nuclei coming in from the embryo sac seemed to have a cumulative effect. Corroboration of this phenomenon was recently made by JONES (1919) on another type of starch difference.

The fact that protein is contained in each of these three types of tissue is a further fact that complicates the genetic problems. HOPKINS, SMITH and EAST (1903) found that after four or five years' selection for high protein and for low protein, the high-protein and the low-protein strains had been differentiated physically to such a degree that the embryos and the amount of corneous starch in the high-protein strain were considerably greater than in the low-protein strain. In a single selected low-protein ear the pericarp and remains of the vestigial glumes comprised 6.67 percent, the endosperms 83.73 percent, and the embryos 9.59 percent of the total. Similarly, in a high-protein ear the maternal tissue comprised 7.71 percent, the endosperms 80.37 percent, and the embryos 11.93 percent. The nitrogen was very low in the maternal tissues and probably did not consist largely of proteid nitrogen. The actual percentage of protein in the embryos was high but did not differ very much in the two types, being 19.91 percent in the low-protein ear and 19.56 percent in the high-protein ear. The greatest difference came in the endosperm, where the aleurone layer (probably contaminated with starch) of the low-protein ear contained 19.21 percent protein as compared with 24.58 percent in the high-protein, and the corneous starch (37.15 percent of the seeds by weight) contained 8.12 percent in the low-protein as compared with 10.99 percent in the high-protein ear (44.89 percent of the seeds by weight).

It is not certain what relative changes in the kinds of protein contained were made by the isolation of these strains, but from OSBORNE and CLAPP'S (1908) analyses of ordinary maize and maize from the high-protein plot of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, one would suppose that the proteins of the endosperm had been increased to a greater degree than the proteins of the embryo.

These various facts regarding the origin of the maize seed and the composition of its various parts have been kept in mind, and the problem of changing the protein content has been attacked in various ways. The results obtained will be discussed seriatim.

It is to be understood that all chemical determinations were made by the methods approved by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

Variation in the protein content of individual seeds

The method of work at the ILLINOIS AGRICULTURAL EXPERIMENT STATION was founded on the fact that in certain ears tested, triplicate samples of three rows of seeds taken throughout the length of the ear gave very uniform analyses, though rather marked variations in analysis were noted when ears were sampled at the tip, middle and butt. Under the circumstances under which the Illinois analyses were made this was perhaps to be expected. It is not surprising, for example, to find variations in the chemical constitution of the seeds at the tip, the middle and the butt, for the seeds in these various regions differ in average size, are pollinated successively from butt to tip because of the maturation of the silks in this order, mature at slightly different times, and presumably may be expected to receive somewhat different amounts of nourishment from the parent plant owing to the spike-like mode of development of the ear. For the same reasons, it is to be expected that samples taken throughout the length of the ear will more truly represent the whole ear. Duplicate samples taken in this manner should be similar. At the same time it should be remembered that analyses of duplicate samples taken in this way, tell one nothing concerning the variation shown by individual seeds or the individual potentialities they carry.

For actual use, no better method of selection can be suggested, yet if individual seeds do show a notable variation due to varying zygotic composition, it will depend largely on the heterozygosity or homozygosity of the genes present, whether progress by the mass-selection method will be rapid or slow. In other words, of two ears of 15 percent protein selected for their high protein content, the one might have a coefficient of variation of 7 percent, the other of 16 percent. In mass selection the ear uniformly high in protein would undoubtedly give the best results, for it is likely that there would be 7-percent or 8-percent seeds, both in a phenotypic and a genotypic sense, in the ear with the high dispersion coefficient. The effect of pollen from the plants these seeds would produce can easily be imagined. On the other hand, if self-pollination were practised, the seeds from the more variable ear would hold out the greatest hopes for improvement, for it is probable they would run as high as 19 or 20 percent.

These suppositions can be illustrated from results actually obtained by analysis of individual seeds. In 1907 the ILLINOIS AGRICULTURAL EXPERIMENT STATION kindly sent some ears of their high-protein and low-protein strains to the CONNECTICUT AGRICULTURAL EXPERIMENT

STATION. These strains after the ten years' selection had already become markedly differentiated. The high-protein strain as compared with the low-protein strain had smaller seeds, larger embryos and a much greater percent of corneous starch. A cross was made between two plants, high-protein female by low-protein male, and a series of seeds of the following types were analyzed: (1) seeds from one ear each of selfed high-protein and of selfed low-protein, these being ears from sister plants of those used in the cross, (2) seeds from the ear produced by the immediate cross, i.e., F_1 seeds, and (3) seeds from each of two F_1 ears bearing F_2 seeds. These analyses were made for another purpose at the time, hence some data which might be useful at the present time are lacking; but they will serve our purpose. Protein contents are calculated to dry basis as has always been our practice, but it was impractical in this case to actually dry each sample by the laboratory method. The ears were air-dried in a steam-heated room, and a single moisture determination made for each ear. Moisture determinations made in this way were about 8.3 percent. Since the variation was small, the range being 1.2 percent, it may be assumed that the method was very accurate for our purpose. The samples were taken in spiral fashion around the ear, as fair a method as could be devised. The results are shown in table 8, where the frequency distribution of protein is tabulated in one-half percent classes.

Because of the small number of individuals and large experimental error involved, and because one cannot feel certain that analyses from a single cross represent accurately the conditions usually found in similar crosses, one should be careful not to draw any hard and fast conclusions from these data. The ranges of variation in the various distributions seem to be rather small, and thus corroborate HOPKINS's conclusions cited previously. The F_2 seeds, however, show a somewhat greater range. Turning to table 9 where the statistical constants are shown, one is somewhat surprised to find the rather high variability of the low-protein type. Judging from the appearance of the curves plotted from the figures given, it seems reasonable to suppose that with larger numbers a greater difference in variability between the parents and the F seeds would be found; but, of course, this is a mere surmise. One conclusion, at least, is permissible. It is clear to anyone who has had experience in studying dispersion coefficients of pedigree cultures, that the phenotypic differences between seeds on the same ear are not what would have been expected had the genotypic differences of these seeds been expressed in the composition of the individual grains. In other words, only a very

TABLE 8

Variations in protein content in individual seeds (dry basis).

Source of seeds	Class centers in percent																				
	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5
Ear A1, high protein....											1	1	7	13	18	14	11	4	1		
Ear B1, low protein.....	1	4	8	22	23	8	5	2	1												
Ear (A2 × B2), F$_1$ seeds										2	4	7	13	26	13	5	1	2			
Ear (A2 × B2)1, F$_2$ seeds									1	0	1	8	4	6	8	18	15	3	2	1	1
Ear (A2 × B2)2, F$_2$ seeds								1	2	2	5	7	11	17	16	12	7	3	1		

small portion of the potentialities which a seed may have inherited is expressed in the quality of the individual seed itself by the time it has finished its growth preparatory to the resting period.

The obvious general conclusion from the data thus far considered, therefore, is this: The seeds of maize vary in the transmissible hereditary factors which control the development of the various proteids in the different parts of the seed. These factors, transmitted in the usual manner, must be the basis of all change in composition through breeding. At the same time, there are extraordinary practical difficulties involved. The greater part of the protein is contained in the endosperm, where, though nuclei presumably duplicating those which form the embryo come together, there is the difference that the contribution from the maternal side is twice that of the paternal side. In addition the composition of the seed as a whole is dependent on extremely complex conditions. First, all external factors affecting growth, play their part; second, the position of the ear, the position of the seed, the size of the seed, and other physiological and morphological factors on which development depends cannot be overlooked; and third, the genetic composition of the plant on which the seed matures, differing as it may from the genetic composition of the seed itself, is of great importance. We cannot differentiate and measure all these influences, but the data already presented are sufficient to show that they exist. When one considers the fact that selection must be founded on the average phenotypic differences shown by fraternities of seeds, and that these phenotypic differences are brought about by such complex conditions, he is constrained to admit that theories as to the effect of selection cannot be based on such material.

TABLE 9

The statistical constants of the frequency distributions shown in table 8.

Source of seeds	Number	Mean	Standard deviation	Coefficient of variation
Ear A1, High-protein	70	13.63±0.06	0.78±0.04	5.72±0.33
Ear B1, Low-protein	74	8.32±0.06	0.72±0.04	8.65±0.48
Ear (A2 × B2), F_1 seeds	73	12.91±0.06	0.78±0.04	6.04±0.34
Ear (A2 × B2)1, F_2 seeds	68	13.71±0.09	1.12±0.06	8.17±0.47
Ear (A2 × B2)2, F_2 seeds	84	12.08±0.08	1.08±0.06	8.94±0.47

There is a possibility, however, of showing some of the effects of particular conditions, and this we shall attempt to do.

TABLE 10

A comparison between the protein content of self-pollinated (S.) and of open-pollinated (O.P.) ears.

Pedigree		1912	1913	1914	1915	1916	1917	1918	Average	
14–30–4–3–7–11–18	S.	12.03	15.23	14.77	15.64	15.08	13.29	15.35	14.89	S.
	O. P.	—	13.46	14.78	14.44	13.11	13.47	15.33	14.10	O. P.
14–30–4–4–2–7	S.	12.03	15.23	14.77	16.13	14.26	14.59		14.99	S.
	O. P.	—	13.46	14.78	13.65	12.17	13.74		13.19	O. P.
14–30–6–11–3–11	S.	12.03	15.23	15.87	16.50	16.90	15.93		16.30	S.
	O. P.	—	13.46	11.79	15.59	15.29	15.27		14.49	O. P.
14–30–6–2–13–5	S.	12.03	15.23	15.87	16.22	14.88	—		15.55	S.
	O. P.	—	13.46	11.79	15.44	12.76	14.20		14.10	O. P.
14–30–6–4–3–13	S.	12.03	15.23	15.87	16.96	15.80	—		16.38	S.
	O. P.	—	13.46	11.79	15.63	10.56	14.10		13.10	O. P.
14–30–9–8–1–6	S.	12.03	15.23	14.51	14.47	15.78	—		14.92	S.
	O. P.	—	13.46	11.60	13.08	11.53	11.12		12.07	O. P.
14–30–12–14–1–10	S.	12.03	15.23	14.26	16.20	14.68	—		15.05	S.
	O. P.	—	13.46	12.13	16.31	12.09	13.81		13.51	O. P.
14–4–6–16–2–12–47	S.	12.03	14.58	14.16	13.53	14.25	14.05	15.27	14.31	S.
	O. P.	—	13.30	12.71	12.42	14.89	14.34	15.00	13.78	O. P.
14–4–6–4–7–8	S.	12.03	14.58	14.16	15.36	14.26	14.14		14.59	S.
	O. P.	—	13.30	12.71	14.65	13.79	14.96		14.47	O. P.
14–4–15	S.	12.03	14.58	13.29					—	S.
	O. P.	—	13.30	—					—	O. P.
14–4–20	S.	12.03	14.58	13.50					13.50	S.
	O. P.	—	13.30	11.97					11.97	O. P.
14–4–1	S.	12.03	14.58	14.60					14.60	S.
	O. P.	—	13.30	12.87					12.87	O. P.
14–6–20–10–3	S.	12.03	—	13.01	14.09	15.93	—	—	14.34	S.
	O. P.	—	—	11.61	12.69	15.09	16.75	16.96	13.13	O. P.
14–22–15–1–29	S.	12.03	13.42	15.86	14.47			15.83	14.90	S.
	O. P.	—	13.47	12.60	13.04			14.56	13.42	O. P.
14–8–11	S.	12.03	14.41	13.96					14.19	S.
	O. P.	—	13.20	13.33					13.27	O. P.
20A–8–5–35–66	S.			16.09	16.49	18.01	16.93	18.69	17.32	S.
	O. P.			13.39	14.90	17.30	—	15.27	15.22	O. P.
20A–4–25–47–24	S.			16.09	15.71	16.15	16.06	16.53	16.13	S.
	O. P.	—		13.39	13.90	14.43	—	16.75	15.03	O. P.
20A–11–10–13	S.			16.09	15.39	16.84			16.12	S.
	O. P.			13.39	14.85	17.01			15.93	O. P.
21–13–9–7–57–43	S.		10.24	10.76	9.61	7.67	7.80	7.39	8.91	S.
	O. P.		9.87	7.09	7.20	8.22	7.38	7.30	7.84	O. P.
21–13–2–11–36	S.		10.24	10.76	11.18	10.01	—		10.60	S.
	O. P.		9.87	7.09	8.15	7.36	9.98		7.76	O. P.
30–1–10–8–3	S.			11.64	13.82	14.44	14.40	16.23	14.72	S.
	O. P.			—	12.57	13.15	14.25	15.09	13.77	O. P
30–7–5–10–7	S.			11.64	13.38	13.03	13.06	13.99	13.37	S.
	O. P.			—	11.91	12.28	12.04	13.85	12.52	O. P.
30–15–4–7	S.			11.64	13.29	14.28	13.80		13.79	S.
	O. P.				12.45	13.80	13.83		13.36	O. P.
Average.........	S.		13.58	14.28	14.65	14.57	13.45	14.91	14.24	S.
Average.........	O. P.		12.66	12.16	13.31	13.05	13.25	14.14	13.10	O. P.
Percent decrease of O. P. below S. is 8.01.								Difference	1.14	

Comparison of the protein content of self-pollinated and of wind-pollinated ears

As we shall have occasion to speak both of wind-pollinated ears—called open-pollinated ears—and of self-pollinated ears, in several connections, let us first compare the difference in proteid content observed under the two systems. Table 10 lists 23 families which have been grown from self-pollinated seed for varying periods of from 3 to 7 years.

The number in the left-hand column is the pedigree number for the last year grown. It follows a system we have long used. The first number, 14–30–4–3–7–11–18. means that plant 18 was the daughter by self-pollination of ear 11 of the generation before, granddaughter of ear 7, and so on. If a cross had been made, it would have been indicated by the multiplication sign.

Each year a number of plants of each strain were self-pollinated. The resulting ears were analyzed, and the average protein content listed as "S." The number of ears obtained varied from one to twenty, but in general from five to ten may be taken as the number used. In addition a composite sample of the wind-pollinated ears of the same strain was analyzed. These figures are designated "O. P."

It will be noted that there is a rather constant difference in protein content in favor of the selfed ears. In only one instance, family 14–4–6–4–7–8– (1917), does the protein content of the open-pollinated ears exceed that of the self-pollinated ears by an amount greater than might be expected to arise from experimental error. In this case the difference in favor of open-pollination is 0.82 percent. Occasionally a rather large advantage—over 4 percent—is held by the self-pollinated ears, but on the average it is 1.14 percent,—the grand average of the self-pollinated ears being 14.24 percent and that of the open-pollinated ears 13.10 percent. In general then one may figure that the protein content of a self-pollinated ear must be reduced by 8 percent of the total amount found to make it comparable with the protein content of an open-pollinated ear.

Just why the open-pollinated seed runs consistently lower than the self-pollinated seed in protein is not wholly clear. The only constant differentials are the use of the paper bag and the application of the pollen all at one time in self-pollination, and one would hardly expect so great an effect from these seemingly inconsequential factors. There are two other differentials of a variable nature, however, which undoubtedly account for a considerable proportion of the difference. In the first place the open-pollinated seeds, though related strains have been planted together, may be expected to show more or less heterosis. And, as we shall show later, heterosis increases the weight of the seed and decreases the percent of protein. This phenomenon accounts for a certain range in the proteid differences and for the correlation between self-pollination and high protein; but it does not answer the whole question, for where heterosis is at a maximum in controlled artificial pollinations, as judged by increase in seed weight, the difference in protein content is only about half of that under discussion. Perhaps the remaining difference is due to a relation between composition and the number of seeds produced. In general, though not invariably, the self-pollinated ears contain a less number of seeds, and HAYES and GARBER (1919) have recently noted that there is a high degree (60 percent) of inverse correlation between number of seeds and protein content. Other things being equal, ears with a large number of seeds have a lower protein content than ears with a small number of seeds.

The immediate effect of pollination on the size and composition of the seed

By taking advantage of the phenomenon of xenia, so-called, a number of interesting facts connected with the composition of maize have been discovered. The procedure has been to select two varieties which differ in endosperm color, and in which the endosperm color of the F_1 seed is not the same as that of either parent. It is obvious that a mixture of approximately equal quantities of pollen may be made from two such plants, A and B, and applied to either plant. Distinguishable selfed and crossed seeds will be obtained on the same ear, maturing under a constant environment.

The effect of crossing on weight of seed in sixteen such mixtures is shown in table 11. Strain A is Illinois Low Protein, a white dent; strain B is Stadtmueller's Leaming, a yellow dent selected for high protein through six generations. Sixteen pairs of plants were selected. The respective mixtures were made and applied to each plant from which the mixture of pollen came. On the resulting ears the selfed and the crossed seeds could be separated easily and weighed. The result was that in each of the 32 comparisons the crossed seeds weighed more than the selfed seeds. The average of A × B over A was 3.7 ± 0.23 cg; and the average of B × A over B, 5.9 ± 0.37 cg. There was a percentage increase of 15.3 in the first case, and 24.2 in the second case.

The difference in actual elaboration of material is even greater than these figures show, for in 25 of the 32 cases listed the water content of the crossed seeds is lower than the selfed seeds (see table 12). The differences are not striking, it is true, but they are large enough to be significant. The average decrease in water content of A × B from A is 0.57 ± 0.09 percent; and in the case of B × A and B, is 0.25 ± 0.08 percent.

The cause of the smaller amount of moisture in the air-dried crossed seeds, is not clear. It has been our experience that it is impossible to dry out immature seeds as well as seeds that have matured normally on the plant. But the fact is that the larger seeds resulting from crosses often appear to mature more slowly than selfed seeds borne on the same ear. Consequently the conclusion that the crossed seeds owe their lack of water to a more rapid and complete maturity is admissible only as a possibility. We are inclined to attribute the matter merely to a difference in the physical constitution of the tissues formed, particularly those of the endosperm. If the pericarp is somewhat more porous and the cell walls within the endosperm somewhat thinner in the crossed seeds, rapidity of drying certainly would be facilitated.

TABLE 11

The immediate effect of pollination upon the weight of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Weight of seeds in centigrams				Parent plant B
		A	A × B	B × A	B	
1	21–13–9–7–57–1	27.0	32.1	30.3	22.3	14–30–4–3–7–11–4
2	–2	20.3	21.9	25.2	21.4	–3
3	–3	26.0	31.1	30.9	22.5	–10
4	–5	22.2	24.3	31.4	25.3	–2
5	–7	26.9	31.1	35.2	28.3	14–30–4–4–2–7–6
6	–10	27.8	32.4	29.9	23.7	14–30–4–3–7–11–1
7	–14	28.0	30.3	39.4	29.5	14–30–4–4–2–7–3
8	–20	30.9	35.5	21.6	21.1	14–30–6–11–3–11–3
9	–24	28.5	33.0	29.1	25.5	14–4–6–4–7–8–5
10	–25	24.6	29.7	36.6	30.1	14–4–6–16–2–12–8
11	–29	32.4	38.4	24.1	19.3	14–30–4–3–7–11–7
12	–31	14.7	17.3	24.3	20.5	–8
13	–33	16.5	18.9	23.6	18.5	–9
14	–35	19.2	23.6	31.3	25.5	–18
15	–36	22.3	25.1	36.4	28.9	14–30–4–4–2–7–14
16	–43	20.6	22.7	34.5	27.3	–2
Average		24.2	28.0	30.2	24.4	
Increase			3.7±0.23	5.9±0.37		
Percent increase			15.3	24.2		

The immediate effect of pollination upon protein content (table 13) is slight. Only 7 instances out of 32 show an increase of protein in the crossed seeds, and there is on the average a decrease that is probably significant; but the decrease is small. The average of A × B is 0.14 ± 0.04 percent less than the average of A; and the average of B × A is 0.60 ± 0.07 percent less than B. When one remembers that the crossed seeds are larger than the selfed seeds, and that the increase in size is greater in B × A than in A × B, it is clear that there is actually more protein produced in the crossed seeds than in the selfed seeds (see table 14). The result of crossing, therefore, is not merely to increase the size of the seed, while the total amount of protein remains the same. The evidence, as far as one may judge from the protein content, is that the increase consists of tissue having practically a normal constitution. At the same time it must be admitted that the increase due to crossing is not uniform in the various parts of the seed, and therefore the increase of one type of protein may be greater than another. This matter is illustrated by the data set forth in table 14.

TABLE 12

The immediate effect of pollination upon the water content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Percent of water in seeds				Parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	7.20	6.90	7.05	7.70	14-30-4-3-7-11-4
2	-2	7.40	6.90	6.80	7.65	-3
3	-3	7.80	7.70	7.22	7.25	-10
4	-5	7.80	7.33	7.13	7.60	-2
5	-7	7.40	6.90	7.15	7.25	14-30-4-4-2-7-6
6	-10	7.18	6.85	6.70	7.55	14-30-4-3-7-11-1
7	-14	8.27	7.55	7.60	7.10	14-30-4-4-2-7-3
8	-20	8.23	6.78	6.65	7.40	14-30-6-11-3-11-3
9	-24	7.45	6.58	6.95	6.95	14-4-6-4-7-8-5
10	-25	7.30	6.83	7.10	6.45	14-4-6-16-2-12-8
11	-29	7.45	7.65	7.32	7.35	14-30-4-3-7-11-7
12	-31	7.30	7.37	7.05	7.20	-8
13	-33	7.53	7.65	7.45	7.30	-9
14	-35	8.45	7.33	7.00	7.55	-18
15	-36	8.48	7.20	7.15	7.25	14-30-4-4-2-7-14
16	-43	8.40	7.05	6.50	7.20	-2
Average		7.73	7.16	7.05	7.30	
Decrease			0.57±0.09	0.25±0.08		

Five pollen mixtures of the kind illustrated in tables 11, 12 and 13, though not the same ones, were used in obtaining A × B, A, B × A and B seeds in which the weights of pericarp, endosperm, and embryo were determined (table 15). The seeds were soaked in boiling water for about 10 minutes, the parts were separated, and dried to a comparatively constant weight in a steam-heated room by exposure to the air in open sacks for one month. The weights of the 10 to 50 seeds comprising each lot were then averaged. The results serve to explain some of the differences obtained in the reciprocal crosses in protein content.

TABLE 13

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Low-protein parent plant A	Percent of protein in seeds				High-protein parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	7.54	7.25	12.64	13.27	14-30-4-3-7-11-4
2	-2	7.16	7.25	11.80	13.06	-3
3	-3	6.98	6.71	12.33	12.81	-10
4	-5	7.46	7.55	12.59	13.19	-2
5	-7	7.49	7.58	14.95	15.90	14-30-4-4-2-7-6
6	-10	7.21	6.71	13.13	13.73	14-30-4-3-7-11-1
7	-14	7.23	7.10	13.59	14.53	14-30-4-4-2-7-3
8	-20	8.59	8.45	16.00	15.93	14-30-6-11-3-11-3
9	-24	8.51	8.23	14.30	14.51	14-4-6-4-7-8-5
10	-25	7.01	7.31	13.72	13.63	14-4-6-16-2-12-8
11	-29	10.47	9.95	11.60	12.08	14-30-4-3-7-11-7
12	-31	7.35	7.02	11.77	12.12	-8
13	-33	7.30	7.45	12.03	12.88	-9
14	-35	6.89	6.61	13.58	14.74	-18
15	-36	7.17	6.94	14.00	14.22	14-30-4-4-2-7-14
16	-43	6.89	6.99	14.11	15.23	-2
Average		7.58	7.44	13.26	13.86	
Decrease			0.14±0.04	0.60±0.07		
Percent decrease			1.85	4.33		

The average weight of the A × B embryos over the A embryos was only 6.14 percent, as compared with the increase of 28.20 percent by which the B × A embryos exceeded the B embryos. In other words the large embryos characteristic of the high-protein strain were increased only 6.14 percent when crossed with the pollen of the low-protein strain, although the small embryos of the low-protein strain were increased by 28.20 percent. Yet this is somewhat of a distortion of the results due to the difference in size of the embryos in the parent strains. As a matter of fact there was no significant difference between the size of the embryos in reciprocal crosses though perhaps this result might have been expected because of a possible difference in the metabolic efficiency of the plants of the different strains upon which the seeds matured. But A × B gave embryos averaging 3.11 cg in weight, and B × A yielded embryos averaging 3.00 cg in weight,—as nearly an even result as could be expected.

In the case of the endosperms, where two maternal nuclei unite with one paternal nucleus, the result was different. The large endosperm of the low-protein type, 27.75 cg, was increased to 32.41 cg by the cross with A; but the endosperm of the high-protein type, 21.55 cg, was increased to 27.27 cg by B pollen. Thus, though the increase of B × A over B was 4.66 cg or 16.79 percent, the increase of A × B over A was 6.02 cg or 27.93 percent. The reciprocals were not alike, as in the case of the embryos. The B × A endosperms exceeded the A × B endosperms by 5.14 cg. Nevertheless, it should be noted that the single nucleus coming from the low-protein (B) increased the endosperm of the seeds borne on A by 6.02 cg, while the single nucleus of the high-protein (A) increased

TABLE 14

The immediate effect of pollination upon the amount of protein in maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Amount protein in centigrams				Parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	2.04	2.33	3.83	2.96	14-30-4-3-7-11-14
2	-2	1.45	1.59	2.97	2.80	-3
3	-3	1.82	2.09	3.81	2.88	-10
4	-5	1.66	1.84	3.95	3.34	-2
5	-7	2.01	2.36	5.26	4.50	14-30-4-4-2-7-6
6	-10	2.00	2.17	3.93	3.25	14-30-4-3-7-11-1
7	-14	2.02	2.15	5.35	4.29	14-30-4-4-2-7-3
8	-20	2.65	3.00	3.46	3.36	14-30-6-11-3-11-3
9	-24	2.42	2.72	4.16	3.70	14-4-6-4-7-8-5
10	-25	1.72	2.17	5.02	4.10	14-4-6-16-2-12-8
11	-29	3.39	3.82	2.80	2.33	14-4-30-4-3-7-11-7
12	-31	1.08	1.21	2.86	2.48	-8
13	-33	1.20	1.41	2.84	2.38	-9
14	-35	1.32	1.56	4.25	3.76	-18
15	-36	1.60	1.74	5.10	4.11	14-30-4-4-2-7 -14
16	-43	1.42	1.59	4.87	4.16	-2
Average....................		1.86	2.11	4.03	3.40	
Percent increase.............			13.1	18.5		

TABLE 15

The immediate effect of pollination upon the weight of different parts of the maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Parent plant A	Parts of seed	Weight in centigrams				Parent plant B
		A	A × B	B × A	B	
14-30-4-3-7-11-5	Embryo	2.68	2.57	2.63	2.10	21-13-9-7-57-6
	Pericarp	2.21	2.57	1.92	2.07	
	Endosperm	21.74	26.79	30.35	24.85	
	Per. and end.	23.95	29.36	32.27	26.92	
	Total	26.63	31.94	34.91	29.02	
14-30-6-2-13-5-1	Embryo	3.04	3.10	2.29	1.82	21-13-9-7-57-9
	Pericarp	—	—	—	1.96	
	Endosperm	—	—	—	22.11	
	Per. and end.	26.71	29.93	29.31	24.08	
	Total	29.75	33.04	31.60	25.91	
14-30-6-2-13-5-11	Embryo	3.30	3.37	3.10	2.26	21-13-9-7-57-15
	Pericarp	2.95	2.99	1.89	1.71	
	Endosperm	22.46	25.32	30.19	25.96	
	Per. and end.	25.41	28.31	32.08	27.68	
	Total	28.71	31.69	35.19	29.94	
14-30-12-14-1-10-4	Embryo	3.05	3.18	2.15	1.92	21-13-9-7-57-22
	Pericarp	1.92	2.15	2.17	1.89	
	Endosperm	21.46	29.08	22.01	22.15	
	Per. and end.	23.39	31.23	24.18	24.04	
	Total	26.44	34.41	26.34	25.96	
14-30-4-4-2-7-7	Embryo	2.62	3.33	4.83	3.64	21-13-9-7-57-39
	Pericarp	2.55	2.93	2.37	2.27	
	Endosperm	20.55	27.91	47.12	43.68	
	Per. and end.	23.10	30.85	49.50	45.95	
	Total	25.72	34.18	54.33	49.60	
Average embryo		2.93	3.11	3.00	2.34	
Increase		0.18=	6.14%	0.66=	28.20%	
Average pericarp		2.40	2.66	2.08	1.98	
Increase		0.26=	10.83%	0.18=	5.05%	
Average endosperm		21.55	27.27	32.41	27.75	
Increase		6.02=	27.93%	4.66=	16.79%	
Average pericarp and endosperm		24.51	29.93	33.46	29.73	
Increase		5.42=	22.11%	3.73=	12.54%	
Average total		27.44	33.05	36.47	32.08	
Increase		5.61=	20.40%	4.39=	13.68%	

the endosperm of the seeds borne on B by only 4.66 cg. There is a discrepancy between the effects of the two pollens for which we have no adequate explanation, though some light is thrown on the matter by the following computations. If one assumes that the heterotic effect would be the same in both crosses and that disregarding this effect the potentiality of each nucleus entering into the formation of the endosperm is realized, then the resulting endosperm of A × B should be one-third of the sum of 21.55 cg plus 21.55 cg plus 27.75 cg, or 23.62 cg, and the resulting endosperm of B × A should be one-third of 27.75 cg plus 27.75 cg plus 21.55 cg, or 25.68 cg. The endosperm actually produced in the cross A × B was 3.65 cg, or 15.5 percent, above the calculated endosperm, and the endosperm actually produced in the cross B × A was 6.73 cg, or 26.2 percent, above the calculated endosperm. The effect of heterosis was presumably greater, therefore, in the case where the low-protein type was the mother.

The increase in the pericarp may be explained as a simple stretching with resulting increase of tissue formation, due to the larger endosperm which had to be covered. Where the increase in endosperm was large, the increase in the pericarp was large; where the increase in endosperm was small, the increase in pericarp was small.

Another rather interesting experiment was made with some of the same pollen mixtures used in obtaining the results set forth in tables 11, 12 and 13. These combinations of pollen from high-protein (variety 14) and from low-protein plants (variety 21) were applied to the silks of a third variety of high-protein plants (20A). One cannot compute the amount of change in seed size, moisture content or protein content when compared with selfed seeds of parent plants C (20A), but a comparison can be made between the effects of the pollen of the low-protein type, 21, and the high-protein type, 14, on the high protein type 20A. Since strain 20A and strain 21 originally came from the same commercial variety of white dent corn, and strain 14 is a yellow dent, it cannot be assumed that the total amount of genetic difference between 20A and 21 is greater or less than the total amount differentiating 20A and 14. For this reason there is no *a priori* justification in predicting a greater or smaller heterotic effect of either pollen on the third strain. The experiments actually did yield a slightly larger seed when the pollen from strain 14 was used. The difference was 1.4 ± 0.38 cg (table 16). If this may be taken to be the result of heterosis, it is a rather important fact, for table 17 shows that there is no significant difference between the moisture content of the two crosses, yet there is a slight but real excess of protein in favor of strain 14,—a difference of 0.26 ± 0.05 percent.

Whether one is justified in generalizing from so few data is problematical. At least it is permissible to point out a possible inference. Illinois

High Protein (20A) and Illinois Low Protein (21), during the process of selection to change the composition, have been differentiated physically in a very marked degree. They must differ by a great many hereditary factors. Stadtmueller's High-Protein Leaming, except for the yellow color of the seeds, resembles Illinois High Protein much more than the latter resembles Illinois Low Protein. Yet the indication of the heterosis test is that Illinois High Protein is genetically more distinct from Stadtmueller's High-Protein Leaming than it is from Illinois Low Protein in certain factors essential to optimum development of the seed. Thus, while it is undoubtedly true that selection for high protein in varieties of different origin does bring about a certain phenotypic uniformity and possibly a considerable genotypic uniformity, nevertheless different combinations of genes contributing toward high-protein complexes may be isolated, and through their union a notable heterotic effect obtained without reduction in protein content other than that due to increased size and number of seeds.

TABLE 16

The immediate effect of pollination upon the weight of maize seed as shown by out-crossed seeds resulting from application of some of the same pollen mixtures used in tables 11, 12 and 13 to a third high-protein variety. Plants grown in 1917.

Pollen mixture number	Parent plant C	Weight of seeds in centigrams		Parent plant A	Parent plant B
		C × A	C × B		
1	20A-8-5-35-8	20.5	24.5	21-13-9-7-57-1	14-30-4-3-7-11-4
2	-3	19.7	23.7	-2	-3
3	-4	25.4	25.0	-3	-10
6	-11	20.3	22.9	-10	-1
8	-24	27.3	27.5	-20	14-30-6-11-3-11-3
9	-26	25.9	27.7	-24	14-4-6-4-7-8-5
13	-6	20.2	20.1	-33	14-30-4-3-7-11-9
16	-13	20.1	25.8	-43	14-30-4-4-2-7-2
16	-15	23.9	27.5	-43	-2
16	-18	21.6	20.9	-43	-2
16	-21	20.2	18.9	-43	-2
16	-30	21.7	21.2	-43	-2
16	-37	21.6	21.0	-43	-2
17	-7	21.3	22.8	-9	14-30-6-2-13-5-1
Average....................		22.1	23.5		
Difference..................			1.4±0.38		

Some further information of a character similar to that just discussed, is given in table 18. Mixtures of pollen from pairs of plants designated

A and B were applied to the plants designated A. The comparison was made by self-pollinating plants B. The table shows the calculations of the percent increase in weight of the crossed seeds over the selfed seeds, and the percent of protein in samples of the selfed and the crossed seeds.

The percent increase in weight of seed in crosses of high protein with high protein vary a great deal, all the way from − 7.30 to 29.23 percent. In at least two of the examples, the small amount of increase is due to variable time of pollination, since small crossed seeds were found only at the tip of some of the ears. But there is also evidence of a real difference in the ratio of increase. For example, the amount of increase when plants of family 14 were pollinated by pollen from plants of family 20A is much greater than when the reciprocal cross was tried. This result is in harmony with the figures obtained when reciprocal crosses were made between families 21 and 14, where the increase was usually greater when the plants of family 14 were used as maternal parents. The explanation would appear to lie in the fact that families 20A and 21 were both originated from Burr's White by selection for high protein and for

TABLE 17

The immediate effect of pollination upon weight, water content and percent of protein in maize seed as shown by out-crossed seed resulting from some of the same pollen mixtures as used in tables 11, 12 and 13. High-protein plants pollinated by a mixture of a distinct high-protein and a low-protein strain.

Pollen mixture number	Parent plant C	Weight of seeds in centigrams		Percent water in seeds		Percent protein in seeds	
		C × A	C × B	C × A	C × B	C × A	C × B
1	20A–8–5–35–8	20.5	24.5	7.25	7.16	16.98	17.30
2	–3	19.7	23.7	7.40	7.25	16.61	16.98
3	–4	25.4	25.0	7.48	7.28	16.35	16.52
6	–11	20.3	22.9	7.10	7.20	17.16	17.65
8	–24	27.3	27.5	7.67	7.63	15.70	15.70
9	–26	25.9	27.7	7.50	7.05	16.36	16.98
13	–6	20.2	20.1	7.30	7.18	13.75	13.74
16	–13	20.1	25.8	6.85	7.00	16.58	17.00
16	–15	23.9	27.5	7.00	6.95	16.33	17.07
16	–18	21.6	20.9	6.98	6.98	12.90	12.77
16	–21	20.2	18.9	7.10	7.00	11.98	12.24
16	–30	21.7	21.2	7.20	7.10	13.34	13.33
16	–37	21.6	21.0	7.40	6.95	13.37	13.37
17	–7	21.3	22.8	7.05	7.44	17.69	18.03
Average		22.1	23.5	7.23	7.16	15.36	15.63
Difference			1.4±0.38	0.07±0.04			+0.26±0.05

low protein respectively. They seem to have retained the power to affect the plants of family 14 somewhat similarly in spite of their present differences in physical appearance.

TABLE 18

Immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed grown upon the same ears (A and A × B seeds from same ears). Plants grown 1916.

	Parent plant A	Percent protein in seeds			Parent plant B	Percent increase in weight of crossed seeds above selfed
		A	A × B	B		
High by low	14-30-9-8-1-3	14.84	14.63	10.63	21-13-2-11-18	16.94
High by high	14-4-6-4-7-26	13.78	13.82	16.22	20A-4-25-37	19.44
	14-4-6-16-7-27	13.14	12.59	16.63	-45	29.23
	-30	12.99	13.04	16.63	-45	8.90
	-28	13.92	13.94	16.63	-45	25.00
	20A-4-25-41	15.95	16.08	14.10	14-4-6-4-7-23	15.29
	-27	16.10	16.53	15.30	-8	−7.30*
	-31	15.61	15.56	16.49	14-30-9-8-1-4	17.48
	-37	16.22	16.27	12.90	14-4-6-4-7-7	6.97
	-45	16.63	16.41	14.44	-24	12.69
	-33	16.69	16.69	16.49	14-30-9-8-1-4	9.91
	-22	16.51	17.20	16.27	-6	−6.86*
	20A-11-10-22	15.65	15.59	—		6.99
	20A-4-25-32	15.85	16.10	16.49	14-30-9-8-1-4	5.36
Average		15.31	15.37	15.72		11.01
Low by high	21-13-2-11-23	10.28	10.25	12.90	14-4-6-4-7-7	15.32
	-36	7.91	7.79	14.44	-24	12.57
	-26 (1)	10.51	10.28	14.44	-24	15.33
	-24	8.23	8.36	13.06	-13	14.54
	-26 (2)	11.85	11.98	14.44	-24	13.65
	21-13-9-7-22	7.54	7.39	15.85	14-30-9-8-1-8	16.03
Average		9.39	9.34	14.19		14.57

* Crossed seeds at tip of ear.

The increase in size of seed when low-protein plants (family 21) are pollinated with pollen from high-protein plants (family 14) is rather uniform.

The difference in protein content between the crossed and the selfed seeds is not marked. In general it follows the amount contained by the maternal parent.

The protein content of different ears borne on the same plant

In a few instances it has been possible to obtain two selfed ears of similar size on the same plant. Numerous pollinations of this kind were made, but in most cases only one ear developed. As seen by referring to table 19, the difference in protein content is very small with the exception of one plant. Sometimes the upper ear is slightly higher than the lower ear, sometimes the reverse is true. The higher protein content probably follows the ear with the smaller number of seeds. At any rate, position on the plant is not a notable factor in influencing the protein.

TABLE 19

Difference in protein content of ears produced on the same plant through self-pollination. Plants grown 1917.

Plant number	Percent protein in seeds			
	Top ear	Bottom ear	Difference	
21–13–9–7–57–54	7.24	6.97*	−0.27	
−58	7.74	8.09	+0.35	
−73	8.72	8.67	−0.05	
−100	11.06	10.91	−0.15	
21–13–2–11–36	7.84	7.91	+0.07	
−26	11.85	10.51	−1.34	
Average..............	9.08	8.84	−0.24	2.64% decrease

* The analysis of this ear was given as 13.79 percent and was not checked as it should have been. This is a difference of 6.53 percent on the same plant. This is nearly twice as large as the greatest difference between any two plants grown the same year in this strain, so it seems that the analysis is wrong. The percent of nitrogen was given as 2.02. In another similar wrong-appearing analysis which was checked, the nitrogen was given as 2.30 when in all probability it should have been 1.30. It therefore seems reasonable to suppose that in this case also the figure should be 1.02 instead of 2.02 and has been so calculated.

The immediate effect of pollination on different ears borne on the same plant

Since two ears borne on the same plant appear to have potentially the same protein contents provided the development of each is similar, the experiments reported in tables 20, 21 and 22 are in a sense repetitions of the experiments reported in tables 13 and 17. There is this point of difference, however: The data for tables 20, 21 and 22 were not obtained by mixing pollen of pairs of plants, applying the mixture to the silks of a single ear, and separating the resulting crossed and selfed seeds by inspection, but by applying foreign pollen to the silks of one ear and self pollen to the silks of another ear on the same plant. Conceivably there might be a difference in the results of the two experiments. In the mixed-pollen experiments a large number either of crossed or of selfed seeds developing on a single ear might influence the size or composition of the seeds of the other type. In the development of two ears having different male parents on the same plant, such influence—if any—would be expected to be small. Under such an hypothesis, there should be a greater difference between the seeds A and the seeds A × B in the two-ear experiments than in the mixed-pollen experiments.

TABLE 20

Immediate effect of pollination upon the protein content of the seeds as shown by selfed and crossed ears borne upon the same stalks (A and A × B are different ears on same plant). Plants grown 1916.

High-protein parent plant A	Percent protein in seeds			Low-protein parent plant B
	A	A × B	B	
14–30–9–8–1–2	16.30	12.57	9.91*	21–13–2–11–18
14–6–20–10–7	17.24	13.88	7.28	21–13–9–7–1
14–4–6–4–7–25	14.58	14.43	11.08	21–13–2–11–31
14–4–6–16–7–15	14.00*	15.43	6.97	21–13–9–7–37
7–14	14.00*	12.76	6.97	–37
2–27	14.25*	15.61	7.84	–40
14–30–9–8–1–5	15.78*	16.24	11.69	21–13–2–11–28
14–6–20–10–9	15.93*	17.30	8.79*	21– –y
20A–11–10–16	16.84*	18.28	12.94	10–4–3–4–5–2–1
20A–8–5–43	18.01*	17.55	8.56	21–13–9–7–5
–42	18.01*	17.21	8.56	–5
–17	18.01*	17.27	7.67*	–10
–37	18.01*	16.97	7.67*	–10
–41	18.01*	16.94	7.67*	–23
Average.............	16.36	15.89	8.83	
Low-protein parent plant A				High-protein parent plant B
21–13–2–11–5	10.23	9.44	17.58	20A–8–5–10
–38	12.32	12.81	18.44	–22
Average.............	11.28	11.13	18.01	

* No selfed ear obtained, average of all selfed ears of the same strain used instead.

It does not seem to us that such a conclusion is justified by the facts, though there are a few instances where the difference in protein content between the selfed and the crossed seeds is notably large. The difficulty in the matter is the paucity of evidence. On the plants of table 20, attempts at self-pollination were successful in only 5 out of 16 cases, owing to a variety of circumstances. No selfed ears being obtained from the plants on which the crosses were, a comparison was made between the percent of protein in the cross-pollinated ears and that of the average of all selfed ears of the same strain. This comparison gave results comparable to those obtained with the mixed pollen. The protein content followed that of the maternal parent, though it lagged somewhat behind. Nevertheless, one could not maintain that a selfed ear obtained on the particular plant used would have had exactly the same value as the average of all the self-pollinated sister ears. Hence a strict comparison is invalid.

TABLE 21

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed produced on different ears on the same stalk. Plants grown 1915.

Low-protein parent plant A	Percent protein in seeds				High-protein parent plant B
	A	A × B	B × A	B	
21–13–2–2	10.52	11.23	15.29	15.92	20A–4–6
2–12	12.19	12.33	15.37	—	–2
2–6	9.24	8.51	—	—	–1
9–10	10.51	8.59	17.50	—	–9
2–5	12.77	12.68	—	16.12	14–30–6–11–9
2–8	12.35	12.13	—	16.24	–10
2–3	11 08	11.87	—	—	2–6
9–4	10.14	11.02	—	—	11–5
9–3	10.40	11.55	—	—	2–1
9–7	7.81	7.47	—	16.86	–3
9–1	10.38	10.35	16.38	—	20A–11–2
Average..........	10.67	10.70			

The five remaining cases comprised three plants where the cross was high protein by low protein, and two cases where it was low protein by high protein. Plant 21–13–2–11–38 crossed with pollen from plant 20A–8–5–28 was the only example of a crossed ear having a higher percent of protein (12.81) than a selfed ear (12.32). In the remaining ears the percent of protein in the crossed ears was less than in the selfed ears, and in two cases the difference is extreme. Plant 14–30–9–8–1–2 selfed had 16.30 percent protein in the ear; but when crossed with pollen from plant 21–13–2–11–18 from a sib averaging 9.91 percent for selfed ears, the crossed seeds contained only 12.57 percent protein. Likewise plant 14–6–20–10–7 yielded a selfed ear containing 17.24 percent protein, but when crossed with pollen from plant 21–13–9–7–1 (7.28 percent protein selfed), the F_1 seeds contained only 13.88 percent protein.

These differences in favor of selfed ears were so much greater than in any of the similarly produced ears, that it is difficult to accept them as correct. On the other hand such differences may be obtained at times, partly for the reasons outlined previously and partly because of the possiblity that certain seed factors may have an immediate influence in particular crosses which is very different from their effect in others.

The theory of factor complexes having different effects seems the more reasonable in view of the results tabled in table 21. There comparable figures are listed for 11 experiments. That is, the figures for the protein of a plant of group A, in this case low protein, were always obtained from a selfed ear of that group, and the figures for the cross of the general formula A × B were always obtained from a second ear borne on the same stalk. The difference in protein content between the crossed ears and the selfed ears is on the whole negligible. Furthermore, considering the data for each pair of ears separately, the crossed ears show an excess in 5 instances and the selfed ears an excess in 6 instances.

TABLE 22

The immediate effect of pollination upon the protein content of maize seed as shown by two ears borne on the same plant one crossed by high the other by low protein. Plants grown 1916.

High-protein parent plant A	Percent protein in seeds				Low-protein parent plant B	High-protein parent plant C
	A × B	A × C	B	C		
20A-4-25-46	18.14	17.77	11.48	15.30	10-3-7-5-4-2-1	14-4-6-4-7-8
20A-4-25-18	16.98	17.17	9.55	16.27	21-13-2-11-20	14-30-9-8-1-6
Average.........	17.56	17.47	10.57	15.79		

In two experiments we were able to obtain a pair of ears on a stalk, the one crossed by high-protein pollen, the other by low-protein pollen (table 22). In the first instance the ear produced by the crossing with the high-protein pollen was slightly higher in protein than the one crossed with low-protein pollen; in the other case the reverse phenomenon occurred.

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants of the same strain

In connection with the work done to estimate the immediate effect of various matings on the size and protein content of the seed, where environmental factors were largely eliminated, it is interesting to examine the results listed in table 23. Here, it is true, environmental factors have full play as far as this is possible on uniform plots where the precautions usually taken in comparative field test are observed. Nevertheless averages of selfed ears and of crossed ears yielded figures of just about the comparative values that would have been expected from the previous work.

The first three reciprocal crosses reported were between various selections of Illinois Low Protein (21) and Stadtmueller's High-Protein Leaming (14). The fourth cross was between Stadtmueller's High-Protein Leaming and Illinois High Protein (20). Each ear, 168 in all, was hand-pollinated.

TABLE 23

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants. Last four pure strains grown 1912, remainder 1913.

Pedigree number	Condition of seeds	Number of ears analyzed	Percent protein in populations	
			Range	Average
(21–3)	Selfed	12	8.94–11.24	10.24
(21–3 × (14–10)–30)	Crossed	4	9.06–10.83	9.72
(14–10)–30 × (21–3)	Crossed	12	12.31–16.71	14.69
(14–10)–30	Selfed	10	13.14–16.22	15.23
(21–3)	Selfed	12	8.94–11.24	10.24
(21–3) × (14–11)–8	Crossed	4	9.55–11.05	10.12
(14–11)–8 × 21–3	Crossed	7	13.35–15.15	14.21
(14–11)–8	Selfed	11	12.78–15.92	14.41
(21–2)	Selfed	15	7.72–12.57	9.41
(21–2) × (14–10)	Crossed	6	8.76–13.37	11.42
(14–10) × (21–2)	Crossed	10	8.74–14.37	11.66
(14–10)	Selfed	14	8.21–15.94	12.19
(14–11)	Selfed	13	8.52–17.86	11.85
(14–11) × (20–2)	Crossed	10	7.73–13.28	10.92
(20–2) × (14–11)	Crossed	10	10.36–16.89	15.10
(20–2)	Selfed	19	11.95–17.10	14.87

In the first two crosses the average protein content of the crossed ears was somewhat lower than the average of the ears of the maternal parent in every case. In the last two crosses the maternal parents were higher than the crosses in two cases. Our conclusions should really be based on the first two crosses, however, for the analysis of the pure strains of the last two crosses was made from ears grown the previous year.

It was thought unnecessary to report the analysis of each individual ear since in no case were more than 15 ears tested, making standard deviations rather untrustworthy. The averages and the protein range of the ears analyzed are sufficient to show that the protein content of the crosses followed that of the mother, and to indicate that the variability of the ears bearing the F_1 seeds was no greater than in the selfed strains.

The protein content of first-hybrid-generation plants bearing second-hybrid-generation seeds

When one compares the protein content of the seeds borne on plants of the first hybrid generation with that of the pure strains from which they came, naturally there is no chance to eliminate variations due to environment except by growing them under as uniform conditions as possible. There is the further difficulty of comparing the actual populations whose plants furnish the gametes for the cross with the hybrid plants themselves. One of three courses may be pursued. Samples of the true parental populations may be held over a year with resultant loss of vitality in the seeds. Parental populations of one year may be compared with hybrid populations of the next year. Or, self-pollinated daughters of the actual parental populations may be tested at the same time as the hybrid populations. The last course of procedure holds some practical advantages, and is probably not any more inaccurate than the other two because of the uniformity of the inbred parental strains.

The first two tests of this kind were made in 1912, one a cross between two high proteins, the other a low protein by a high protein.

Strain 20–2, Illinois High Protein, ranged from 11.95 percent to 17.10 percent protein in the 19 selfed ears analyzed, with an average of 14.87 percent. It yielded at the rate of 39.7 bushels per acre. This strain was crossed with No. 14, of which two selections grown in 1912 yielded at the rate of 50.1 bushels per acre. The first, 14 (1911 seed), ranged from 8.52 percent to 17.86 percent in the 13 ears analyzed,—an average of 11.85 percent. The second selection, 14 (1910 seed), ranged from 8.21 percent to 15.94 percent (14 ears),—an average of 12.19 percent. The F_1 plants, 20–2 × 14–11, yielded at the rate of 55.1 bushels per acre, and ranged from 9.25 percent to 15.02 percent (12 ears),—an average of 11.85 percent. The parental average in protein was thus 13.45 percent, while the average of the F_1 plants was 1.60 percent lower.

Illinois Low Protein (21–2), ranging from 7.70 percent to 12.57 percent in protein (average 9.41 percent for 16 ears), and yielding 42.3 bushels per acre, was also crossed with strain 14. The result was an F_1 generation yielding 53.3 bushels per acre, with ears ranging from 6.24 percent to 13.03 percent protein (24 ears). The average of the F_1 generation, 9.18 percent, was therefore 1.54 percent below the parental average.

In 1915 some further comparisons between F_1 generations and parental strains were made based upon analysis of hand-pollinated selfed ears. Cross 14–6 × 21–13 was the union of Stadtmueller's High-Protein Leaming (14.09 percent average) and Illinois Low Protein (10.40 percent average). The ears of the F_1 plants averaged only 9.49 percent protein but the cross was particularly vigorous, and yielded at the rate of 112 bushels per acre. The remaining F_1-generation plants tested this year were all high-protein matings. The results are recorded in table 24. The protein ranges are not given, as they are similar to other homologous cultures reported in the paper. One need only note that with high-protein strains crossed together, the protein in the seeds of the F_1 ears is below that of the parents. The yields of the F_1 plants are so much greater than those of the inbred strains, however, that the protein per acre is much larger.

TABLE 24

Effect of crossing upon protein content as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with self-pollinated ears. Plants grown 1915.

Parent strains		Percent protein; average selfed ears					Yield, bushels per acre
♀	♂	1915 ♀	1915 ♂	1914 ♀	1914 ♂	1915 F_1	F_1
20A–1	14–6	15.86	14.09	16.09	13.01	13.71	90
20A–2	14–6	15.86	14.09	16.09	13.01	14.01	93
14–30–4	14–6	15.89	14.09	14.77	13.01	14.66	46
14–30–12	14–8–11	16.20	14.49	14.26	13.96	14.02	47
14–6	14–30–12	14.09	16.20	13.01	14.26	13.81	80
14–6	20A–1	14.09	15.86	13.01	16.09	13.94	72
Average.........		15.33	14.80	14.54	13.89		71
Average of parents		15.07		14.22		14.03	
Difference between F_1 and average of parents		From 1914 average, −0.19 = 1.33 percent decrease From 1915 average, −1.04 = 6.90 percent decrease					

TABLE 25

Effect of crossing upon protein content and yield as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with open-pollinated ears. Plants grown 1917.

Pedigree numbers		Yield bushels per acre			Percent protein in seeds O. P.		
♀ parent	♂ parent	♀	♂	F_1	♀	♂	F_1
High × high							
20A-4-25-36	14-4-6-4-7	34.6	23.2	108.6	14.21	14.96	13.02
20A-4-25-1	14-30-6-4-3	63.7	25.7	112.6	11.51	14.10	10.66
14-4-6-16-7-28	20A-4-25-45	59.2	67.0	107.9	13.43	14.72	13.10
14-4-6-4-7-26	20A-4-25-37	23.2	55.1	110.8	14.96	13.48	12.36
14-4-6-16-7-27	20A-4-25-45	45.5	67.0	124.2	12.95	14.72	12.98
Average.......		45.2	47.6	112.8	13.41	14.40	12.42
Average of parents......................................					13.91		
Decrease of F_1 below average of parents 10.71 percent.........							−1.49
High × low							
20A-4-6	21-13-2-2	—	—	118.2	—	—	8.30
20A-4-25-18	21-13-2-11	55.1	42.1	105.9	13.48	7.07	9.05
14-30-9-8-1	21-13-2-11	49.1	42.1	127.5	11.12	7.07	8.43
14-30-9-8-1	21-13-2-11	49.1	42.1	(156.4)*	11.12	7.07	8.81
14-6-20-10-7	21-13-9-7-1	20.3	65.2	97.2	16.75	6.63	9.36
Average.......		43.4	47.9	112.2	13.12	6.96	8.79
Average of parents......................................					10.04		
Decrease below average of parents 12.45 percent.............							−1.25
Low × high							
21-13-9-7-5	14-4-6-16-2-7	56.3	45.3	101.6	6.70	11.91	8.41
21-13-9-7-18	14-4-6-16-2-12	71.7	45.5	103.6	6.41	13.99	9.25
21-13-9-7-10	14-4-6-16-2-12	76.6	45.5	122.9	6.21	13.99	8.45
21-13-9-7-7	14-4-6-16-2-12	72.8	45.5	120.2	6.46	13.99	8.09
21-13-2-11-5	20A-8-5-10	55.8	56.3	120.0	7.07	12.93	10.49
Average.......		66.6	47.6	113.7	6.57	13.36	8.94
Average of parents......................................					9.97		
Decrease below average of parents 10.33 percent.............							−1.03

* Calculated from imperfect stand with few plants, probably too high, not included in average.

As we have seen, the protein content of seed matured under bags after hand-pollination is approximately 8 percent higher than that of ears of the same strains after wind-pollination, although the immediate effect of the pollen is negligible. This factor should be taken into consideration when the remaining comparisons between F_1 and parent strains are studied, for these later analyses were all made upon open-pollinated seed.

Fifteen tests were made in 1917,—five each of high protein by high protein, high protein by low protein, and low protein by high protein. The data obtained are in many ways preferable to the comparisons made between hand-pollinated ears. A larger number of ears were used, reducing the error of random sampling; the ears were of a more uniform size; the seeds were more numerous; and the maturation of the seeds was on the whole better. The results, shown in table 25, are simply a corroboration of those obtained earlier.

TABLE 26

Protein content of two high-protein types and of first, second and third seed generations of crosses between them. Analyses made with self-pollinated ears.

Pedigree number	Seed generation	Year grown	Number analyzed	Range of protein percentage	Average of protein percentage
14	P_1	1912	13	8.21–18.95	12.02
(20–2)	P_1	1912	18	11.95–17.10	14.87
(20–2) × 14	F_1	1912	12	9.25–15.02	11.85
[(20–2) × 14]–3	F_2	1913	21	12.39–15.89	14.22
[(20–2) × 14]–8	F_2	1913	16	13.21–16.10	14.83
[(20–2) × 14]–3–14Y	F_3	1914	6	14.70–16.63	15.84
[(20–2) × 14]–3–14W	F_3	1914	5	15.05–16.77	15.96
[(20–2) × 14]–3–15Y	F_3	1914	5	14.75–16.10	15.31
[(20–2) × 14]–3–15W	F_3	1914	6	14.53–16.81	15.33

The similarity of the tests within each quintet, makes it necessary to discuss the averages only. We may note first that the F_1 ears are always lower in protein than the average of the parental strains, and that this decrease is rather uniform. In actual percent protein, it is highest in the "high × high" crosses and lowest in the "low × high" crosses, yet the difference is only 0.46 percent. When the difference is reckoned on the mean percent protein, the situation changes. The "high × low" crosses show a decrease of 12.45 percent of the percent of protein carried by the parents, with the other two classes showing 10.71 percent and 10.33 percent respectively. Averaging the results, gives an expectancy of a decrease of a little over 11 percent in protein below the average of the parents in any cross between inbred types. There is, as PEARL and BARTLETT (1911) and HAYES (1914) maintained, a semblance of a dominance of low protein, but the matter is not so easy to interpret. The percent of protein in the F_1 ears is about the same whether the low protein is the maternal or the paternal parent, and it stands nearer to the low-protein than to the high-protein parent; but when one considers the crosses between high-protein strains, it is evident that this decrease can be interpreted as an effect of heterosis. Consider the yields of the high-protein strains and their hybrids. The extreme vigor of the hybrids causes a yield of more than double the "pure" types. Thus in spite of the lower protein content, the total amount of protein per acre in the hybrids is twice as large as in the parent strains. If then one increases the percent of protein in the "low × high" and "high × low" crosses by 11 percent of the amount found to correct for heterosis, the percent protein in the hybrid would be somewhat closer to that of the high-protein parent (see also table 26).

Conclusions regarding the inheritance of protein in maize

There is some advantage in pausing at this point to bring together the odds and ends of data regarding inheritance of protein in maize, before discussing the remainder of the experiments.

In the first place it is perfectly clear that the external conditions, the factors of environment, have such a marked effect on the protein content of maize that it may be raised or lowered as much as 40 percent above or below the total percent produced under average growing conditions. This conclusion may be drawn from a study of change in direction of the protein curve in the high-protein and the low-protein strains grown by the Illinois Agricultural Experiment Station, from the fluctuations from year to year in our own selected strains, from the difference in protein content between hand-pollinated and wind-pollinated ears belonging to the same strain, and from the protein content of two self-pollinated ears from the same stalk.

No doubt the protein content of maize is affected by each and every environmental factor which has an influence on the development of either the plant as a whole or the seed in particular. For example, some lack of nitrogen might appear to affect the development of the stalk and leaves more than the seeds, and some lack of phosphorus might appear to affect the seeds more than the remainder of the plant, but it seems likely that each plays its part in protein synthesis. These various factors cannot have their influences separated and their individual effects described at present, and it probably would not make matters a great deal clearer if this could be done in the rough manner which would necessarily be inherent in such analysis. The factors of environment work together as do the parts of a machine, and though a greater or smaller degree of efficiency in one part of the machine has its effect, the absence of that part stops the machine. There is one thing that may be emphasized, however. A departure from the optimum temperature and moisture at critical periods of the plant's growth appears to overshadow other features in influencing the constitution of the grain. When some of the other conditions are not at their best a plant produces smaller ears or a less number of seeds without there being any great interference with the normal development of the chemical constituents, but let there come a radical diminution in the available moisture or an extreme temperature change after these organs have been laid down normally, and the effect on development is very great. Nevertheless even under such handicaps, it would seem that nearly the normal amount of protein is developed. The percent of protein is influenced, of course, but it is influenced largely through the diminished elaboration of starch.

Taking these facts as we find them, one can realize what great errors may be made in selection. Seeds due to contain 12 percent protein under a hypothetical "normal" environment, may contain anywhere from 9 percent to 15 percent protein because of the conditions under which they develop; mass selection of desirable phenotypes is therefore of less value than with any other character with which we have had experience.

Admitting that proteid variations in maize are to a great extent due to the modifications imposed by a fluctuating environment, one need only study the work of the ILLINOIS AGRICULTURAL EXPERIMENT STATION to realize what a great rôle heredity plays in the matter. Then comes the important question: Can one estimate the number of differentiating hereditary factors involved and describe the method by which they are inherited? If a precise answer to this question is desired, it must be no. But the situation is not as discouraging as this answer indicates. Some definite conclusions can be drawn which are of real practical value.

The number of hereditary factors affecting protein elaboration by which varieties of maize may differ must be large. This is an indefinite statement, it is true; but what is meant is that the facts will hardly admit the presumption that they may one day be analyzed by the assumption of five or six differentiating determiners. Possibly the main factors involved are some such small number, but apparently there are modifiers that may run into the hundreds.

The evidence in the case is indirect; at the same time, it is valid. In the first place there are the data of OSBORNE showing the complexity of the protein situation. There are several kinds of proteids. The proteids are very different in their chemical nature. And they are distributed throughout the various tissues which go to make up the seed. Second, the work of the ILLINOIS STATION as well as our own investigations show noteworthy physical differences accompanying change in protein content. Numerous different size relations may be obtained between embryo and endosperm, and between the various tissues making up the endosperm. Size of seed may be thirty times as great in one case as in another. Ear size, number of seeds, shape of seeds, etc., each plays its rôle. Third, the ILLINOIS STATION, starting with a single variety previously brought to a considerable degree of uniformity through selection for physical characters, has been nearly twenty-five years isolating their high-protein and low-protein types without coming to the point where there seems to be no hope of further differentiation.

These facts all point to an involved hereditary complex, a large number of multiple factors affecting protein in the species as a whole. On the other hand, the inexact method of work used at the ILLINOIS STATION, makes it unwise to multiply unduly in our imagination the heterozygous factors involved in their material. Such mass-selection experiments might be carried on for many generations without reaching the end desired when only four or five hereditary factors were under consideration. In fact, the great changes in protein content obtained after only two or three generations of selection in our own experiments because of the control of both parents, lead us to believe in a relatively small number of "main" factors. But the number of subsidiary factors,—factors playing minor rôles,—is by no means small.

The mathematical possibilities involved in recombinations of multiple factors (see EMERSON and EAST 1913) is now a matter of common knowledge. Moreover theory has been corroborated by practical results. Tests have been made on scores of animals and plants and the results reported in numerous scientific papers during the past decade. If such a simple scheme of interpretation could be used for the inheritance of protein, at least an outline of the method of transmission could be made without difficulty. But we are confronted with a much more complicated matter than the cases previously described, due to the protein content of the seed being in part in the embryo and in part in the endosperm, as has already been noted. This is a difficulty inherent in breeding all the cereals, yet it is a difficulty that has been overlooked except for passing mention in one or two papers of the senior author.

The basis of all hereditary transmission, in all such breeding work, is of course the zygote which comes into being with the fusion of two gametes, ♀ + ♂; but at the same time that the zygote is formed the endosperm is laid down by three gametes carrying the same qualities, ♀ ♀ ♂. By successive cell divisions the seed is formed. Now 20 percent of the protein of the maize seed is contained in the ♀ ♂ embryo through which all transfer of hereditary qualities is made, while 80 percent of the protein is found in the ♀ ♀ ♂ endosperm which can have no part whatever in hereditary transmission. One can simplify matters to some degree, however, if he keeps in mind that the size of the embryo and the percent of protein it contains were raised but slightly in the early experiments of the ILLINOIS STATION. The notable variations appeared in the endosperm. The problem, therefore, is the mechanism by which a ♀ ♂ embryo transmits characters which are exhibited in a ♀ ♀ ♂ endosperm. Transmission through the zygote presumably is by the usual methods. Gamete formation is typical. Segregation and recombination occur as in other species, and gametes combine to form zygotes by chance.

It would seem as if no argument need be made in favor of the assumption that the seed is the unit and not the ear. The seed is the new organism, and all of our modern biological evidence leads us to suppose that the seed is formed as described above, and that *many different* hereditary possibilities may be contained in the seeds of a single plant. On the other hand this does not preclude the probability that the genetic constitution of the plant on which the seed matures has a marked influence on its size, shape and composition. In a word the phenotype of the seed may be influenced by the mother no matter what is the genetic composition of the individual seed. The uniformity of the seeds of a single ear in shape and size, the comparative lack of variability in composition of the seeds of a single plant lead to this view.

Keeping these fundamental ideas in mind, what conclusions can be drawn from our data?

Only three facts seem to stand out as important. The chemical composition is influenced by heterosis. This influence on the seed is slight but significant, resulting in a somewhat larger size and concurrent decrease in percent of protein. The influence on the seeds borne by hybrid plants is much greater. The plants themselves being more vigorous than those of the parental strains, the seeds they bear are larger and more numerous, and contain a much smaller percent of protein. Second, the influence of the factors borne by a male gamete are practically without immediate influence on the seed they help to form. Third, the protein content of the seeds of an F_1 hybrid, when corrected for the influence of heterosis, is intermediate between that of the two parents with a tendency to be somewhat closer to that of the high-protein parent.

These facts force us to one of two conclusions. Either the prompt reaction of the two maternal nuclei utilized in the inception of the endosperm has a controlling influence on chemical composition; or, the genetic constitution of the mother plant is the major determining factor. We cannot deny an influence to the immediate reactions within the $3x$ endosperm cells of any seed due to their own individual genetic constitution. There is a demonstrable heterosis as an immediate effect of pollination. There is production of pigments,—at least one ether soluble and at least two water soluble. There is change in the physical character of the starch (horny or floury). Therefore the individual genetic constitution of a seed must effect real changes from the very beginning of the life history. One can hardly call these changes radical, however, when compared with those caused by the genetic constitution of the mother plant. There is no adequate reason for supposing the effect of the two maternal nuclei is more than double the effect of the paternal nucleus, and as far as the change in composition is concerned the latter is almost negligible.

By way of parenthesis it may be said here that this conclusion appears to have considerable theoretical importance. BATESON'S work on the inheritance of pollen color and shape, and the work of EAST on pollen color and self-sterility in Nicotiana have shown the genetic constitution of the mother to be the effective agent. The hereditary factors carried by the gametes seem to have no function during the period of gametic generation. They are passive. The activities of the gametes, their size, shape and color, are determined by the mother's complex.

EAST assumed that the individual constitutions of the gametes were negligible during the haploid generation, that their inheritance was held in abeyance until the formation of the zygote, then to come to the fore to play a rôle in the ontogeny of the organisms.

The data cited in this paper, however, appear to point to a delayed use of individual powers, so to speak, even after the zygote is formed. The individuality of the organism seems to gain momentum as the life history progresses. The genetic constitution by which a seed may differ somewhat from its mother, the inherited individuality which it has received, does not become apparent in the early stages of life. The mother still controls during the time the seed is being matured, parasitically as it were, on the body of its parent, and presumably during the early stages of independent life while stored nutriment is still being utilized.

Returning to the subject in hand, let us summarize our conclusions. Besides the practical difficulties arising from the influence of varying factors of environment, maize breeding for seed characters, and the breeding of other cereals as well, is complicated by a most exaggerated lack of correlation between individuality and performance, between phenotype and genotype. One must select by the characters possessed, which are largely influenced by the constitution of the mother plant, yet the characters which the adult plant will possess are determined by the union of nuclei *both* of which may differ widely in potentialities from those possessed by the plant on which it grew.

An example will perhaps make this clear. It is wholly theoretical and diagrammatic. Let us suppose that the differential factors between two plants, a high-protein and a low-protein plant, let us say, are represented by independent factors A, B, C and D. The high-protein plant is AA BB CC DD; the low-protein plant is aa bb cc dd. A cross is made reciprocally. Except for a slight decrease in protein content due to heterosis the composition of the F_1 seeds follow the mother plant. Seeds Aa Bb Cc Dd from the high-protein mother, are high in protein; seeds of the same genetic constitution, Aa Bb Cc Dd, from the low-protein mother, are low in protein. In a general selection experiment (starting with unknown pedigrees) one would undoubtedly breed from the former; one could obtain the same end results by breeding from the latter.

Samples from either of these F_1 populations are grown. The average protein content of the ears produced is about the same,—lower than the average of the pure strains entering into combination,—because of heterosis. The seeds vary individually in their protein content, but most of this variation is due to size, position on ear, etc. Only a small proportion of the variation is due to the genetic constitution of the individual seeds themselves. Aside from variations due to the extraneous causes mentioned, the ears are fairly uniform. The protein content has followed the mother. Yet by ordinary recombination the productive capacity is manifold. There are eighty-one ($= 3^4$) actual classes, counting both homozygotes and heterozygotes. And the same troubles ensue in later generations, though in a somewhat lesser degree.

This illustration gives food for thought in connection with cereal breeding. One realizes just why the work carried on by the Experiment Stations in cereal breeding has been so comparatively unproductive.

Presumably scientific methods have yielded no better results than earlier empirical methods. The reason is not far to seek. The later methods have been just as blind, just as empirical as the former. It is also clear why the workers at the ILLINOIS STATION misinterpreted the effects of selection. Selection was endowed with a creative power because of the length of time close selection without pollen control could be carried on without eliminating hope of further progress, and because dispersion indices were not reduced when determined on population averages of seeds (ears). This study, we hope, has done something toward clarifying matters by pointing out the source of the difficulties. But this is not all. There is a method of breeding which may be followed by which results can be obtained in a much shorter time Some of the indefiniteness and blindness of the Illinois method can be eliminated. By its use we have obtained some rather remarkable increases in protein content in a few generations. Furthermore we were able to keep up, and even to increase the yields of the standard varieties used. In the work at the ILLINOIS STATION, the yields were so greatly reduced through inbreeding that they were unprofitable. Their yields for the last six available years are shown in table 27.

TABLE 27

Yields in the chemical-selection experiments of the Illinois Agricultural Experiment Station, 1913–1918. Bushels per acre.

Year	High protein	Low protein	High oil	Low oil	Control variety
1913	30.2	35.5	31.0	23.9	39.6
1914	36.2	43.3	37.4	48.7	55.2
1915	42.4	57.2	45.2	49.9	53.5
1916	14.6	29.6	16.7	19.8	28.2
1917	48.9	56.3	55.9	51.3	63.9
1918	38.8	47.8	46.6	58.2	62.8

We do not maintain that it is desirable to undertake breeding for high protein, or other chemical constituents, as a practical method of increasing food value or industrial utility, but it can be done in the following manner. Self-pollinate large numbers of plants artificially. Test the seeds produced by each individual as accurately as possible by the progeny-plat method. With the continuation of inbreeding if a large enough series be tested, near-homozygous plants having the ability to produce high-protein seeds will be obtained. Some of the crosses between such types will have a high yield and will retain the power to produce large quantities of protein. To be sure the percent of protein in the vigorous hybrids will not equal the percent in the purified parent strains. But relatively the percent will be high, and actually the protein per acre will be rather remarkable.

EXPERIMENTS ON BREEDING FOR HIGH PROTEIN

Original experiments on selection

Selection experiments after the pattern of those conducted at the Illinois Agricultural Experiment Station, were begun in Connecticut in 1906. Seed was selected and a number of ears analyzed from three standard varieties, one a dent, the other two flint. Frequency distributions constructed from these data are shown in table 28. They are given merely to throw a little additional light on the amount of protein as found in unselected varieties grown in Connecticut.

Table 28

Frequency distribution of the protein in the ears of certain Connecticut-grown varieties of maize. Analyses on open-pollinated ears.

Variety	Class centers in percent of protein														
	Year	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0
Stadtmueller's Leaming...	1906 seed				10	15	6	4	3	2					
	1907 seed						4	6	8	13	10	5	0	0	2
	1908 seed						1	3	12	13	3	6	1	1	
	1908 crop	2	8	12	18	17	20	21	20	13	4	5	1	1	
Hopson's Longfellow.	1906 seed									1	25	11	5	5	1
	1907 seed							5	9	16	11	6	1		
Sturges's Hybrid......	1906 seed						19	14	26	12	12	7	5	1	

Note: Sturges's Hybrid was produced by crossing a North Carolina Dent with King Philip Flint and selecting toward a twelve-rowed flint type. The corn in 1906 after 8 or 10 years of selection was true to the flint type, and 84 ears out of 96 were twelve-rowed. There were 5 with 10 rows, 6 with 14 rows, and one with 16 rows.

No protein selections were made on the variety known as Sturges's Hybrid, which was a twelve-rowed flint. Hopson's Longfellow, an eight-rowed flint, was selected again in 1907, and Stadtmueller's Leaming was grown for three years. The results, omitting details, are found in the table.

It was soon found, from discoveries made in an extended investigation on heredity in maize, that this method of procedure was hopeless. It had no scientific basis, and carried with it no prospects of the production of a variety which would elaborate a high percentage of protein and at the same time give large yields of grain. This work was discontinued, therefore, until 1912 when the experiments on heredity and on the effects of inbreeding and cross-breeding had proceeded far enough to give some idea of the correct methods to pursue. Two lines of breeding were then started, the one a series of selections in self-fertilized lines, the other a series of selections in alternately crossed and selfed lines. They will be described in order.

Selections in self-fertilized lines

Selections in self-fertilized lines were made on four varieties,—Stadtmueller's Leaming, Burwell's Flint, Illinois High Protein and Illinois Low Protein. No great amount of work was done on any one variety, for the expense of such an investigation is considerable and the available resources were small. Nevertheless the data show conclusively that by breeding successively from self-fertilized ears, strains high in protein can be obtained in a relatively short time.

Stadtmueller's Leaming

Table 29 shows the results obtained between 1912 and 1918 on Stadtmueller's Leaming. The data can be followed easily by referring to the number of the "mother ear" planted. Twenty-seven self-pollinated ears were obtained from planting variety No. 14. These were analyzed. The percent protein varied from 8.21 to 17.86, with an average of 12.03. From the ears highest in protein of this population, five lines were started. The first family in the table descended from ear 14–6. The next selection was ear 14–6–20. The second selection was ear 14–6–20–10. From the population produced by this ear in 1916, two ears were grown. One was ear 14–6–20–10–3, grown in 1917; the other was ear 14–6–20–10–15, grown in 1918.

Passing down the table, the second selection from the population of 1912 was ear 14–30. In 1915, the seeds from two sister ears of 1914 were planted. These are numbered 14–30–4–3 and 14–30–4–4. Thus two lines branch off from ear 14–30–4 in that year, and the ancestors of ear 14–30–4–4 can be followed by referring to the family tabled above.

The major extreme in the population of 1912 was an ear containing 17.86 percent protein. If a larger number of ears had been analyzed, an ear still higher in protein might have been expected. It is clear then that a commercial variety unselected for high protein may contain

TABLE 29

Selection of Stadtmueller's Leaming for high protein in self-fertilized lines.

Subject matter	1912	1913	1914	1915	1916	1917	1918
Mother ear planted	No. 14		14–6	–6–20	–20–10	–10–3	–10–15
Protein in mother ear	—		17.86	16.21	15.92	17.30	16.59
Range S.-P. population	8.21–17.86		9.87–16.21	11.82–15.92	13.47–17.30	—	—
No. S.-P. ears analyzed	27		4	11	10	—	—
Ave. S.-P. population	12.03		13.01	14.09	15.93	—	—
Ave. O.-P. population	—		11.61	12.69	15.09	16.75	17.07
Mother ear planted	Ditto	14–30	–30–4	–4–3	–3–7	–7–11	–11–18
Protein in mother ear		15.80	15.59	14.93	16.18	16.47	14.74
Range S.-P. population		13.14–16.22	13.74–15.52	14.17–16.68	13.39–16.47	12.08–15.05	14.10–16.75
No. S.-P. ears analyzed		10	8	11	10	13	9
Ave. S.-P. population		15.23	14.77	15.64	15.08	13.29	15.35
Ave. O.-P. population		13.46	14.78	14.44	13.11	13.47	15.33
Mother ear planted	Ditto	Ditto	Ditto	–4–4	–4–2	–2–7	
Protein in mother ear				15.52	16.64	15.82	
Range S.-P. population				15.37–16.64	12.40–15.92	12.45–15.90	
No. S.-P. ears analyzed				4	7	8	
Ave. S.-P. population				16.13	14.26	14.59	
Ave. O.-P. population				13.65	12.17	13.74	
Mother ear planted	Ditto	Ditto	–30–6	–6–2	–2–13	–13–5	
Protein in mother ear			16.22	16.03	16.16	15.90	
Range S.-P. population			15.18–16.29	14.49–17.39	13.30–15.90	—	
No. S.-P. ears analyzed			4	6	5	—	
Ave. S.-P. population			15.87	16.22	14.88	—	
Ave. O.-P. population			11.79	15.44	12.76	14.20	
Mother ear planted	Ditto	Ditto	Ditto	–6–4	–4–3	–3–13	
Protein in mother ear				15.96	17.45	16.30	
Range S.-P. population				15.65–17.72	15.04–16.30	—	
No. S.-P. ears analyzed				6	7	—	
Ave. S.-P. population				16.96	15.80	—	
Ave. O.-P. population				15.63	10.56	14.10	
Mother ear planted	Ditto	Ditto	Ditto	–6–11	–11–3	–3–11	
Protein in mother ear				16.29	18.33	16.90	
Range S.-P. population				15.31–18.33	—	—	
No. S.-P. ears analyzed				7	1	1	
Ave. S.-P. population				16.50	16.90	15.93	
Ave. O.-P. population				15.59	15.29	15.27	

TABLE 29 (continued)

Subject matter	1912	1913	1914	1915	1916	1917	1918
Mother ear planted	No. 14	14–30	–30–9	–9–8	–8–1	–1–6	
Protein in mother ear	—	15.80	15.63	15.49	14.59	16.27	
Range S.-P. population	8.21–17.86	13.14–16.22	13.28–15.49	12.35–17.46	14.67–16.49	—	
No. S.-P. ears analyzed	27	10	11	8	7	—	
Ave. S.-P. population	12.03	15.23	14.51	14.47	15.78	—	
Ave. O.-P. population	—	13.46	11.60	13.08	11.53	11.12	
Mother ear planted	Ditto	Ditto	–30–12	–12–14	–14–1	–1–10	
Protein in mother ear			16.09	15.63	14.11	16.77	
Range S.-P. population			12.67–15.63	13.73–18.64	12.23–16.77	—	
No. S.-P. ears analyzed			6	9	10	—	
Ave. S.-P. population			14.26	16.20	14.68	—	
Ave. O.-P. population			12.13	16.31	12.09	13.81	
Mother ear planted	Ditto	–14–22	–22–15			–15–1	–1–29
Protein in mother ear		14.83	15.43			15.46	15.14
Range S.-P. population		11.81–15.43	—			12.42–15.66	14.75–16.39
No. S.-P. ears analyzed		16	2			13	13
Ave. S.-P. population		13.42	15.86			14.47	15.83
Ave. O.-P. population		13.47	12.60			13.04	14.56
Mother ear planted	Ditto	14–8	–8–11				
Protein in mother ear		14.75	15.92				
Range S.-P. population		12.78–15.92	13.16–14.61				
No. S.-P. ears analyzed		11	6				
Ave. S.-P. ears analyzed		14.41	13.96				
Ave. O.-P. population		13.20	13.33				
Mother ear planted	Ditto	14–4	–4–6	–6–4	–4–7	–7–8	
Protein in mother ear		14.73	15.48	14.62	16.50	15.30	
Range S.-P. population		11.90–15.77	13.65–14.68	14.25–16.95	12.90–15.30	13.51–14.68	
No. S.-P. ears analyzed		14	10	11	17	11	
Ave. S.-P. population		14.58	14.16	15.36	14.26	14.14	
Ave. O.-P. population		13.30	12.71	14.65	13.79	14.96	
Mother ear planted	Ditto	Ditto	Ditto	–6–16	–16–2	–2–12	–12–47
Protein in mother ear				14.68	'5.62	15.82	14.75
Range S.-P. population				12.20–15.62	11.90–15.82	13.63–14.75	14.71–15.88
No. S.-P. ears analyzed				10	10	10	21
Ave. S.-P. population				14.04	14.25	14.05	15.27
Ave. O.-P. population				12.42	14.89	14.34	15 00

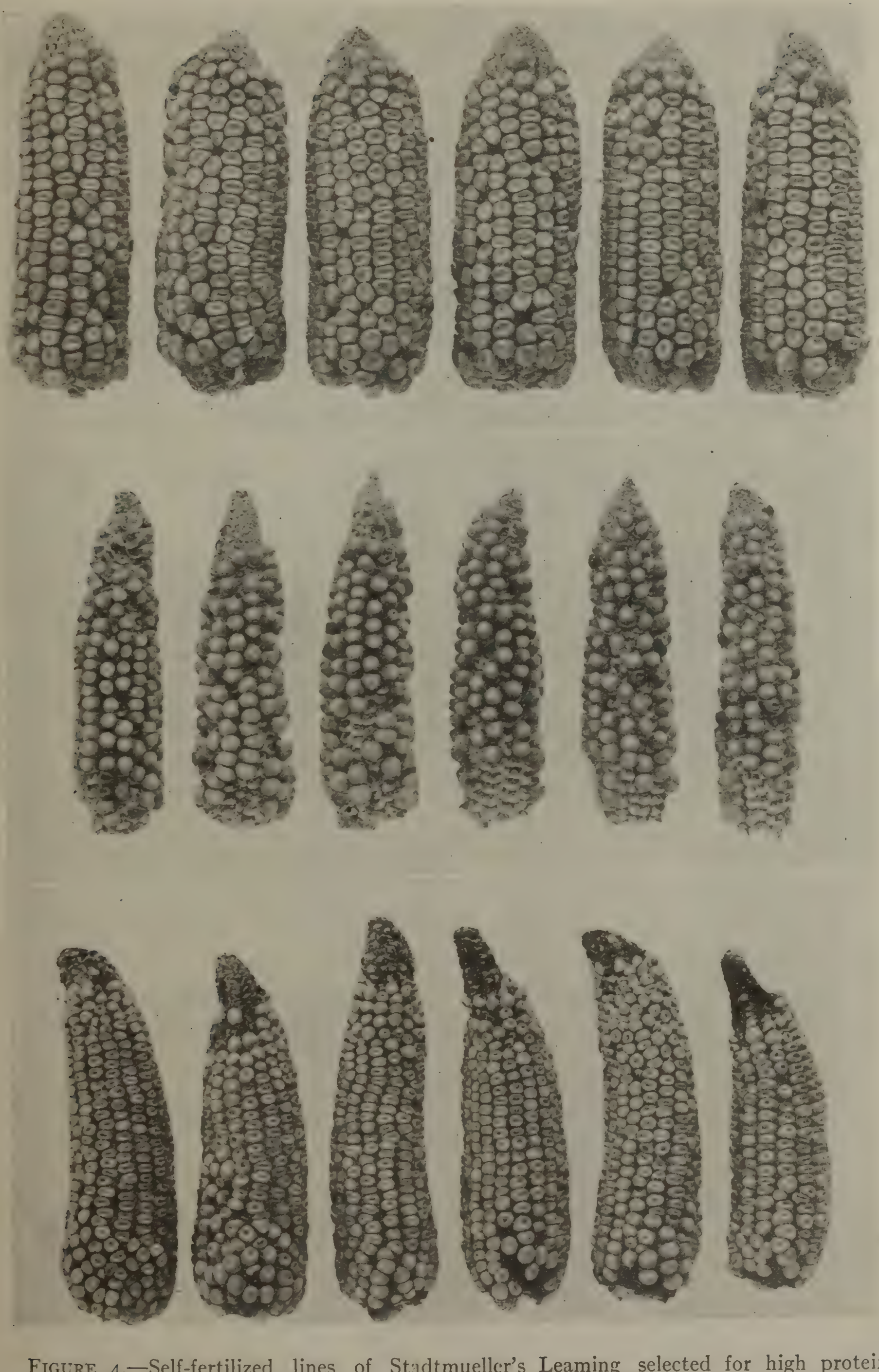

FIGURE 4.—Self-fertilized lines of Stadtmueller's Leaming selected for high protein. Above 14-4; center 14-22; below 14-30.

ears which are very high in protein. And when one considers the fact that these analyses are made on populations of seed in which the parentage of the individual cannot be controlled, there is reason to believe that almost any commercial variety contains hereditary factors which when brought together in a homozygous condition will produce ears as high in protein as those the ILLINOIS STATION has secured after nearly a quarter of a century of mass selection from open-pollinated ears.

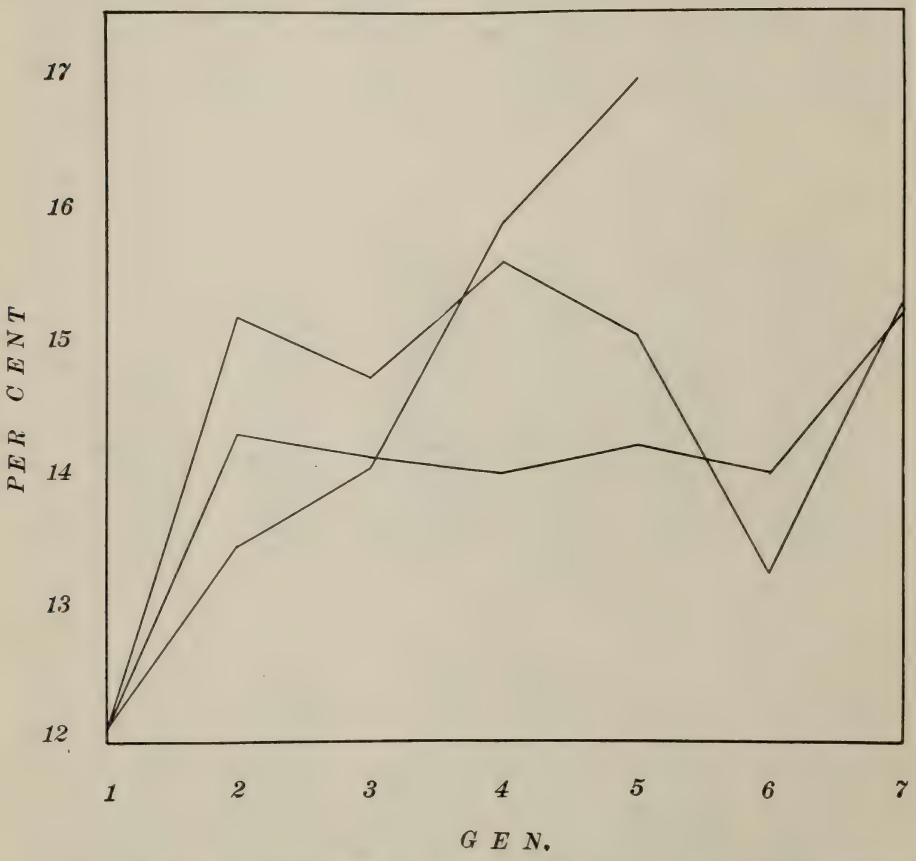

FIGURE 5.—Graphical representation of the results of selecting Stadtmueller's Leaming for high protein in self-fertilized lines.

The highest percentage of protein obtained in any one year's crop at the ILLINOIS STATION was 15.66 in 1916. In 1918 family 14–6–20–10–15 of Stadtmueller's Leaming contained 17.07 percent protein. This amount came as the result of five years of selection. Since the fluctuations from year to year are so wide, one could not rest assured that this strain would continue to produce quite as high a percentage of protein as this; but the fact that it produced 15.09 percent of protein in 1916, and that a sister ear this crop produced 16.75 percent in 1917, makes the quality of performance of this family rather certain.

Perhaps the next best average selection is found in the family ending in ear 14–30–6–11–3–11. The last three years the crops averaged 15.59, 15.29 and 15.27 percent respectively. Some other families did almost as well, but to these two must be given the prizes for protein production. Reference must be made to table 29, if one really wishes to make a comparative study of the strains. Further description in the text is superfluous if this is done; if not, any description is likely to be inadequate.

Illinois High Protein

Selections from the Illinois High-Protein strain were grown at various times between 1906 and 1914 from seed kindly sent to us by Professor L. H. Smith, but not until 1914 were regular analyses made of the ears produced. In that year a mixture of a small quantity of seed from each of a number of ears from the Illinois High-Protein crop of 1913 was grown. Only a few self-fertilized ears were obtained, but of these nine were analyzed. They averaged 16.09 percent, and ranged from 14.97 percent to 16.64 percent. A mixed sample of the open-pollinated ears contained 13.39 percent, which does not indicate a particular aptness for protein production in the Connecticut conditions.

Three selections were grown, two of which were carried on for five years. Table 30 shows the results.

The selfed strains from this material, (including Illinois Low Protein) were better from a developmental standpoint than any of the others included in the experiments. They also reached a comparatively static condition of uniformity more quickly. This was to have been expected, however, for the Illinois strains having been selected through the mother plants rather closely for sixteen generations must have made some approach toward homozygosity in their various characters. Nevertheless it is interesting to note that the experience of the strain during the years of selection in Illinois had not eliminated all possibilities of improvement. In other words, they still exhibited some heterozygosity. This fact may be shown in three different ways. First, there was a reduction in vigor due to the more intense inbreeding of self-fertilization. Second, the two families 20A–4 and 20A–8 are so different from one another that they can easily be distinguished either by the plants or by the ears. No. 20A–8 is smaller in size of plant, earlier to flower, and produces more abundant pollen. The seeds are more corneous and are scarcely dented at all. Third, the advance in protein content from selection in one of the self-fertilized lines is remarkable. The ratio of selective elimination was very small compared to that of the ILLINOIS STATION, for we did not have the facilities for analyzing large numbers of ears; yet, there is no question but that progress in the isolation of a high-protein strain sped more rapidly after selection by this method was begun. Family 20A–8 was different in this respect from family 20A–4. The curve of the latter family shows little change from year to year. Presumably it was more nearly homozygous in the beginning.

TABLE 30

Selections of Illinois High Protein for high protein in self-fertilized lines.

Subject matter	1914	1915	1916	1917	1918
Mother ear planted	20A	20A–8	–8–5	–5–35	–35–66
Protein in mother ear	—	16.64	18.32	18.56	18.60
Range S.-P. population	14.97–16.64	15.32–18.32	17.52–18.97	13.83–18.60	16.35–20.49
No. S.-P. ears analyzed	9	10	11	10	23
Ave. S.-P. population	16.09	16.49	18.01	16.93	18.69
Ave. O.-P. population	13.39	14.90	17.30	—	15.27
Mother ear planted	Ditto	20A–11	–11–10		
Protein in mother ear		16.34	16.57		
Range S.-P. population		13.94–16.57	15.51–18.56		
No. S.-P. ears analyzed		12	11		
Ave. S.-P. population		15.39	16.84		
Ave. O.-P. population		14.85	17.01		
Mother ear planted	Ditto	20A–4	–4–25	–25–47	–47–24
Protein in mother ear		16.27	16.23	16.91	16.31
Range S.-P. population		14.90–16.23	14.93–16.91	15.36–16.63	15.60–17.91
No. S.-P. ears analyzed		10	12	4	22
Ave. S.-P. population		15.71	16.15	16.06	16.53
Ave. O.-P. population		13.90	14.43	—	16.75

Illinois Low Protein

The same argument may be made in the case of Illinois Low Protein. The two families raised came from a single open-pollinated ear of 1912. The two lines separate in 1915. Family 21–13–2–11–36 reaches the year 1917 with a protein content of 9.98 percent, but family 21–13–9–7–57–43 in 1918 had only 7.30 percent protein. The latter family was a rather constant low-protein performer, and if it had been possible to analyze a large number of ears one can hardly doubt a still more rapid drop. The low limit of 6.41 percent obtained in a self-fertilized ear of 1918 is an indication of what might have been expected (see table 31).

It may be worth while to note here that these particular families of Illinois Low Protein were characterized by deficient pollen production. One line was lost because pollen sufficient for producing self-fertilized ears could not be obtained.

Table 31

Selection of Illinois Low Protein for low protein in self-fertilized lines.

Subject matter	1913	1914	1915	1916	1917	1918
Mother ear planted....	21	21–13	–13–2	–2–11	–11–36	
Protein in mother ear..	—	9.24	10.16	8.62	7.91	
Range S.-P. population.	8.94–11.24	10.00–11.50	8.62–14.18	7.91–12.32	—	
No. S.-P. ears analyzed.	12	6	11	15	—	
Ave. S.-P. population..	10.24	10.76	11.18	10.01	—	
Ave. O.-P. population..	9.87	7.09	8.15	7.36	9.98	
Mother ear planted....	Ditto	Ditto	–13–9	–9–7	–7–57	–57–43
Protein in mother ear..			10.80	7.81	6.68	6.89
Range S.-P. population.			7.81–12.81	6.68–10.15	6.89–11.06	6.41–9.28
No. S.-P. ears analyzed.			11	16	20	20
Ave. S.-P. population..			9.61	7.67	7.80	7.39
Ave. O.-P. population..			7.20	8.22	7.38	7.30

Burwell's Flint

It has always been the impression among maize-breeders that flint varieties in general average somewhat higher than dent varieties in protein. In fact both the average of all flint varieties and the maximum for flint varieties are somewhat greater than the average and the maximum for dent varieties in Jenkins and Winton's (1892) compilation. Moreover our own analyses of Hopson's Longfellow show considerably more protein than those of Stadtmueller's Leaming (table 28). Mindful of this fact selection in self-fertilized lines was undertaken with a standard Connecticut variety known as Burwell's Yellow Flint.

The results (table 32) were not as satisfactory as one might wish. In a series of 19 self-fertilized ears, daughters of self-fertilized ear No. 30, there was only a range of from 7.40 percent to 13.68 percent protein. This was not very promising material. The average of 11.64 percent in self-fertilized ears, probably a percent or two higher than those of the open field, was really lower than that of any flint we have analyzed. But as a means of demonstrating the practicability of the method of breeding, one variety was as good as another, and as this variety was above the average in productiveness, it was used.

Three lines were split off in 1915 and continued for four years. Each of the families yielded to selection but not in the same degree. The banner selection was family 30–1 in which the protein content rose continuously at almost a uniform rate. Based on self-fertilized ears, the average protein content rose from 11.64 percent to 16.23 percent, a gain of 4.59 percent of actual protein content or over 40 percent of the protein originally contained in the variety. Since the range of protein in the 10 selfed ears analyzed in this strain in 1918 was low, and since the maximum was greater than had been found previously in the variety, the prospect of obtaining a really efficient protein producer in Burwell's Flint is thus fairly good.

TABLE 32

Selection of Burwell's Flint for high protein in self-fertilized lines.

Subject matter	1914	1915	1916	1917	1918
Mother ear planted.........	30	30–1	–1–10	–10–8	–8–3
Protein in mother ear.......	—	13.68	14.67	16.51	15.36
Range S.-P. population......	7.40–13.68	12.74–14.67	12.08–16.51	12.96–15.56	15.81–17.00
No. S.-P. ears analyzed......	19	10	9	10	10
Ave. S.-P. population.......	11.64	13.82	14.44	14.40	16.23
Ave. O.-P. population.......	—	12.57	13.15	14.25	15.09
Mother ear planted.........	Ditto	30–7	–7–5	–5–10	–10–7
Protein in mother ear.......		13.48	14.36	14.14	13.78
Range S.-P. population......		11.39–14.36	11.70–14.14	12.24–13.78	13.10–15.31
No. S.-P. ears analyzed......		10	10	10	13
Ave. S.-P. population.......		13.38	13.03	13.06	13.99
Ave. O.-P. population.......		11.91	12.28	12.04	13.85
Mother ear planted.........	Ditto	30–15	–15–4	–4–7	
Protein in mother ear.......		13.24	14.28	15.85	
Range S.-P. population......		12.59–14.28	12.68–15.85	13.17–14.97	
No. S.-P. ears analyzed......		10	10	8	
Ave. S.-P. population.......		13.29	14.28	13.80	
Ave. O.-P. population.......		12.45	13.80	13.83	

Conclusions regarding selection for high protein in self-fertilized lines

MENDEL'S original paper showed that the result of self-fertilization without selection on any allelomorphic pair Aa is to reduce the number of heterozygotes so that in the nth generation the ratio is $1 : 2^n - 1$. Equal fertility for all plants and random mating of gametes is of course assumed.

EAST and HAYES (1912) generalized this expression, for independent inheritance showing that the probable number of homozygotes and of any particular class of heterozygotes is expressed in the formula

$[1 + (2^r + 1)]^n$, where r is the segregating generation and n is the number of allelomorphic pairs. JENNINGS (1916) stated the same formula somewhat differently.

In 1917 JENNINGS considered the numerical results of breeding when genes are linked, and found that while the formulae are complex, the general result in self-fertilization is to decrease the number of heterozygotes and to increase the number of homozygotes.

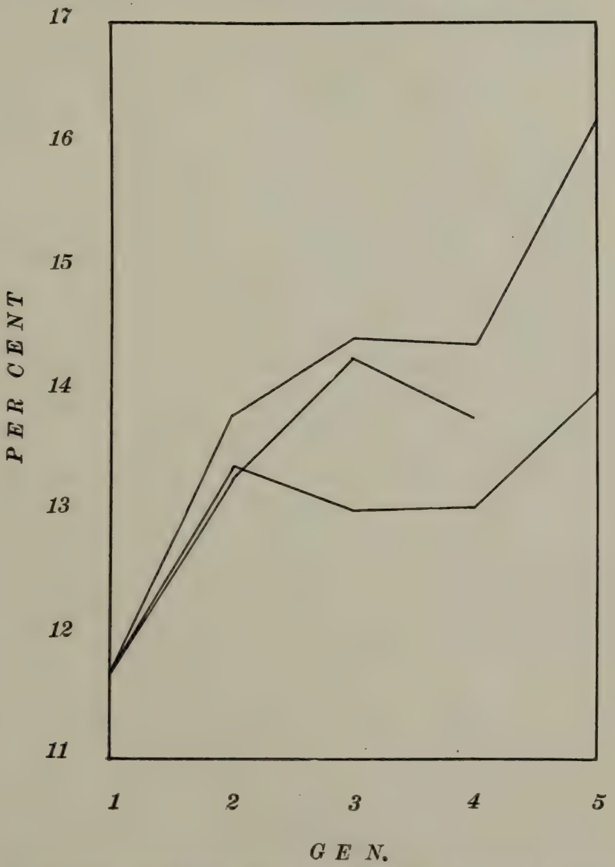

FIGURE 6.—Graphical representation of the results of selecting Burwell's Yellow Flint for high protein in self-fertilized lines.

More recently JONES (1918), by studying the variability of various characters in maize in successive self-fertilized generations, has demonstrated that these mathematical generalizations actually hold in practice.

The data presented here are merely a corroboration of these results on another character complex. There are indeed many complications in the inheritance of protein, as has been emphasized in the preceding pages.

Nevertheless, it can be stated without hesitation that methods of self-fertilization will give high-protein strains of maize in shorter time than any other procedure. We need only call attention, therefore, to one or two practical points in connection with the strains thus produced.

In the first place, there is no direct correlation between protein content and heterosis. In fact, the correlation, if any, is negative. In other words, inbreeding for protein does not have to overcome the obstacles which stand in the way of breeding for yield in that yield is a function of vigor and vigor dependent to some extent on heterosis. On the other hand, inbreeding reduces the vigor, and with it the size of the ear, the size of the seeds and the number of the seeds per ear. Since there is an inverse correlation between size and number of seeds and protein content, the percent of protein actually found in our inbred strains is higher than may be expected in high-yielding strains with the same potential protein production. The same statement holds for such closely bred strains as those produced by the long-continued selection experiments of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, though there the vigor has not been depressed as it would have been by a like number of generations of self-pollination. The facts being as they are, however, we must take them into consideration in any practical method of breeding for high protein, for in order to have any desirability whatever in agricultural practice a strain of maize or any other crop must have a high yield. Without the power of yielding high returns, no commercial variety can survive, be its particular qualities what they may. To try and surmount these difficulties, several experiments were undertaken where the strains under observation were alternately selfed and crossed.

Selected matings between high-protein plants

One of the major difficulties in producing high-protein strains by inbreeding is the lack of a practical method for making rigid selections based upon large numbers. In other words straight selection on a small scale does not begin to exhaust the possibilities inherent in such a variable cross-fertilized plant as maize. Theoretically, one should test out all the extreme individuals in a very large population; but this is impracticable. A partial solution of the problem was found, however, in a plan by which selected high-protein plants were crossed together.

This plan is based upon the plausible assumption that since the various inbred high-protein strains differ in their morphological features, similar protein percentages may be due to different genetic constitutions. The

TABLE 33

Effects of crossing and selection upon protein content 1914. Analyses of cross-pollinated ears between inbred strains of high-protein corn. Plants grown 1914.

Plant number ♀	×	Plant number ♂	Percent protein	Crossed ears planted 1915
14–4–1–6	×	14–30–9–7	14.66	
–10	×	14–6–2	13.23	
–8	×	14–30–12–8	14.67	
–4	×	14–6	13.42	
–3	×	14–6–1	14.09	
–9	×	14–30–4–16	14.07	
–12	×	14–8–11–2	13.75	
–15–12	×	14–8–11–4	13.32	
–5	×	14–6–7	12.89	
30–4–16	×	14–4–1–9	12.10	
–6	×	(20 × 14)–3–14W–1	12.90	
–12	×	14–6–13	15.54*	E
–6–8	×	14–6	13.93	
–9–3	×	14–6–3	13.80	
–7	×	14–4–1–6	11.53	
12–10	×	14–6–17	13.10	
12–8	×	14–4–1–8	12.55	
–16	×	14–8–11–6	15.31*	F
–6	×	14–6	15.02	
14–6–3	×	14–30–9–3	13.78	
–6	×	14–30–12–6	11.89	
–9	×	14–8–11–5	12.99	
–8	×	14–4–1–3	12.77	
–2	×	14–4–1–10	12.31	
–1	×	14–4–1–3	15.38	
–7	×	14–4–15–15	12.59	
–5	×	14–30–6–8	14.83	
–17	×	14–30–12–10	10.71*	G
–15	×	20	13.33	
–16	×	20	9.08*	D
–22	×	(20 × 14)–3–14–12	12.17	
–4	×	(20 × 14)–3–14–1	13.15	
14–8–11–5	×	14–6–9	14.84	
20	×	14–6–15	17.98*	A
	×	14–6–16	18.48*	B
(20 × 14)–3–14–1	×	14	15.27	
–1	×	14–30–4–6	16.37*	C

* Selected for growing 1915.

procedure was simply to cross different selected high-protein lines, to self-pollinate the first-generation plants, and to select again from the progenies which represent segregating generations. Cross matings were made between individual plants of several such second-generation families in as large numbers as possible, using each plant both as a male and as a female. Since it had already been determined that the immediate effect of pollination was small, the analysis of the seed was used to indicate the value of the individual in reciprocal crosses. Thus the crosses selected for continuation by self-fertilization were those in which both parents were high-protein performers.

The results from the first year's work carried on in this manner are set forth in table 33. From among the families of Stadtmueller's Leaming (14) and Illinois High Protein (20) which had been selected for high protein in self-fertilized lines during three or more years, thirty-seven crosses were made. The protein contents of the ears produced ranged from 18.48 percent to 9.08 percent,—the minor extreme possibly being an error of analysis. The results of the analyses are practically the same as they would have been had self-fertilized ears of the mother plants listed been analyzed. Nevertheless, since reciprocal pollinations were made in several cases, it is clear that we were dealing with individuals which had the ability to store up rather large quantities of protein. In 1915, seven of these ears were planted.

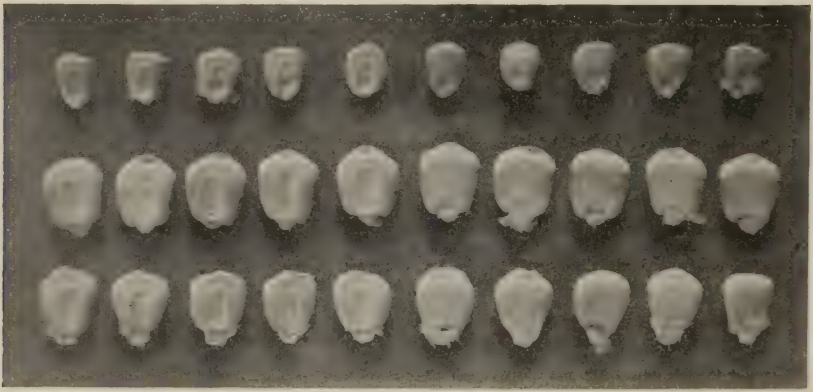

FIGURE 7.—Above, Illinois High Protein; below, Illinois Low Protein; center, F_1 generation.

Table 34 presents the results of analyzing ten selfed ears from the vigorous plants produced by these high-protein extremes. On the whole there is not much choice in the various lots. Lot C with ears ranging from 16.32 percent to 13.99 percent is the best. Its average protein content was 15.33 percent, not a bad average, though 1 percent lower than the parent ear.

Table 34

Effects of crossing and selection upon protein content. Analyses of first-generation crosses between high-protein strains. Plants grown in 1915.

Pedigree number	Designation	Range of percent protein in seeds of individual selfed F_1 plants										Average	Percent protein of parent ear
		Highest ear									Lowest ear		
20 × (14–6–15)	A	15.86*	14.81	14.36	14.26	14.22	14.17	13.71	13.45	12.81	12.46	14.01	17.98
20 × (14–6–16)	B	16.50*	14.81	14.69	14.44	13.59	13.29	13.04	13.04	12.07	11.61	13.71	18.48
(20 × 14)–3–14W–1) × (14–30–4–6)	C	16.32*	16.14	15.78	15.52	15.47	15.14	15.04	14.99	14.88	13.99	15.33	16.37
(14–6–16) × (20)	D	16.38*	15.29	15.08	14.69	14.21	14.10	14.02	12.64	12.41	10.57	13.94	9.08
(14–30–4–12) × (14–6–13)	E	15.92*	15.48	15.08	15.17	14.94	14.63	14.49	13.86	13.68	13.39	14.66	15.54
(14–30–12–16) × (14–8–11–6)	F	15.13*	14.89	14.62	14.59	13.95	13.69	13.38	13.36	13.34	13.23	14.02	15.31
(14–6–17) × (14–30–12–10)	G	15.55*	14.57	14.54	14.52	13.96	13.55	13.52	13.28	12.42	12.20	13.81	10.71

* Selected for growing 1916.

TABLE 35

Effect of crossing and selection upon protein content. Analyses of cross-pollinated ears grown on F_2 plants from the highest F_1 ears. Plants grown in 1916.

Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein
A2 × B2	14.16	B1 × C3	16.65	C3 × B1	14.73	D2 × A3	16.55	E2 × D6	14.26	F2 × D6	17.10	G1 × F2	12.50
A4 × D2	14.77	B2 × A4	18.57*	C7 × B7	14.64	D4 × A6	11.44	E4 × D6	13.30	F3 × D6	15.74	G2 × F2	12.10
A5 × C7	14.88	B3 × C7	16.78	C10 × B7	11.07	D6 × A5	9.76	E6 × D6	13.58	F4 × D6	15.49	G3 × F2	14.86
A6 × D2	14.15	B4 × C10	13.90	C11 × B7	10.75	D9 × B10	9.78	E8 × D4	9.28	F5 × A14	10.93	G15 × F15	10.74
A8 × D2	12.13	B6 × C42	16.82	C13 × B20	14.99	D10 × B10	9.43	E16 × C22	14.26	F13 × A8	14.28	G17 × F15	13.58
A10 × D2	15.41*	B7 × A4	16.22	C22 × B20	11.45	D12 × A5	9.34			F15 × A8	12.68	G19 × F15	10.25
A11 × D2	14.74	B9 × A4	17.70	C23 × B20	18.16*	D15 × A4	15.28			F17 × A8	15.05	G21 × F15	14.88
A13 × D4	15.75	B10 × A23	15.75	C27 × B7	13.39	D18 × A11	15.21			F19 × A8	16.46	G31 × F29	10.73
A14 × D18	11.81	B12 × A23	12.83	C28 × B22	12.21	D21 × A11	16.26			F21 × A8	10.74	G39 × F31	12.10
A15 × D4	17.43	B16 × D15	16.38	C42 × B22	12.01	D22 × A11	14.09			F22 × A8	12.66	G40 × F31	15.08
A17 × D18	11.12	B20 × D15	18.35*			D24 × A11	15.15			F23 × A8	15.79		
A18 × D18	13.43	B22 × C42	11.19			D25 × A15	11.67			F27 × C27	13.14		
A19 × D18	14.56	B27 × A25	17.00			D26 × A15	12.61			F29 × O.P.	12.02		
A22 × B2	15.23*	B28 × A25	16.04			D27 × A15	16.35*			F31 × G32	13.23		
A23 × D18	14.66	B38 × C42	12.62			D29 × G19	15.60						
A25 × D18	13.39	B41 × A46	16.15*			D30 × A18	12.79						
A26 × B2	16.21*					D31 × G31	15.92						
A28 × D21	15.81*					D34 × A4	16.72						
A36 × D18	17.85*					D35 × A36	12.13						
A38 × D30	16.69					D39 × A36	14.79						
A41 × D18	16.07					D41 × A4	16.14						
A46 × D30	15.71					D43 × A41	9.72						
A47 × D35	16.46												
A48 × D35	17.35												
Average	14.99	Average	15.81	Average	13.34	Average	13.49	Average	12.94	Average	13.95	Average	12.68
Parent A	15.86	Parent B	16.50	Parent C	16.32	Parent D	16.38	Parent E	15.92	Parent F	15.13	Parent G	15.55

* Selected for growing 1917 based upon the protein content of both parents.

The major extreme of each lot was planted in 1916, and a series of crosses made between the resulting plants (see table 35). The protein content was determined on 101 of these crosses. As mother plants, lot B proved to be the best,—the average for the ten ears tested being 15.81 percent. The second highest average came from lot A used as mothers, —an average of 14.99 percent based on 24 ears. Two ears having over 18 percent protein were obtained, and ears with over 17 percent were numerous. Those ears marked with an asterisk were planted the spring of 1917.

Several ears were selfed from each of these ten selections. The analytical results are shown in table 36. The columns of the table are in descending order of the protein content of the parent ears. If now we note the order of the average protein content of the offspring we find it is thus: 1, 5, 4, 10, 2, 6, 7, 3, 8, 9. In other words the plus half of the parents produced four-fifths of the plus half of the offspring, and the minus half of the parents likewise produced four-fifths of the minus half of the offspring. The parent-offspring correlation is so high that one cannot doubt the great influence of heredity on the result. The general result is that three strains of corn have been produced after seven years' work, one averaging 16.31 percent, the second averaging 15.42 percent, and the third averaging 15.05 percent in protein. They have considerable vigor, give fair yields of grain, and at the same time are as high in protein as most of the straight self-fertilized families. In these strains, moreover, there is a reserve of genetic variability out of which it is possible theoretically to carry the percent of protein to a still higher level.

The two ears highest in protein content from family (C23 × B20) were planted in 1918 (see table 37). From the plants of lot (C23 × B20) −8, the fifteen selfed ears obtained averaged 17.68 percent protein. The average was thus higher than the parent ear by a slight margin. The majority of the ears were over 18 percent in protein, and one reached the extraordinary figure of 20.14. Here, then, was a fairly high-yielding partially inbred strain which in the single year of 1918 probably averaged over 16 percent in protein in the ordinary field run of ears.

The second lot of plants, daughters of lot (C23 × B20) −6 was not quite as good as the other, still an average for fifteen selfed ears of 16.39 percent protein would have been considered exceptional had the sister strain not been in existence. Four of the fifteen ears were over 18 percent in protein.

Two other lots of seed were grown as checks. No. 170 was a seed mixture taken from the three highest ears of *each* of the ten selections

TABLE 36

Effect of crossing and selection upon protein content. Analysis of selfed ears from high-protein crossed ears in 1916. Plants grown in 1917.

	C23×B20	A26×B2	A22×B2	D27×A15	B20×D15	B2×A4	A36×D18	A28×D21	A10×D2	B41×A46
Percent protein, ♀ parent 1916..	18.16	16.21	15.23	16.35	18.35	18.57	17.85	15.81	15.41	16.15
Percent protein, ♂ parent 1916..	18.35	18.57	18.57	17.43	15.28	14.77	15.21	16.26	16.55	15.71
Average, parents 1916..........	18.26	17.39	16.90	16.89	16.82	16.67	16.53	16.04	15.98	15.93
Percent protein offspring selfed 1917	17.59*	15.31	15.53*	14.31	16.05*	15.99	14.66	16.75*	16.25	16.53
	17.25*	14.63	15.17	13.23	15.99	15.12	14.47	15.59	15.65	13.43
	16.93	14.04	14.99	12.01	15.64	15.01	13.82	15.67	13.89	13.11
	16.52	13.60	14.88	11.01	15.61	13.37	13.27	15.42	13.74	12.65
	16.39	13.42	14.78	10.47	15.58	13.36	13.32	14.71	12.37	11.95
	16.25	14.95	14.45	8.89	15.48	13.42	13.18	14.58	12.16	11.92
	16.18	12.94	14.26		15.23	12.89	12.76	14.57	12.13	11.90
	16.09	12.72	13.10		15.02	11.04	12.14	14.22	11.96	11.27
	16.00	12.08	12.44		14.91	10.85	11.79	13.92	10.53	10.65
	13.90	10.58	11.56		14.72	9.87	9.20		9.58	10.32
Average offspring 1917........	16.31	13.43	14.12	11.65	15.42	13.09	12.86	15.05	12.83	12.37
Ordinal relation 1917.........	1	5	4	10	2	6	7	3	8	9

* Selected for planting 1918.

TABLE 37

Effect of crossing and selection upon protein content. Analyses of selfed plants of the progenies of two ears highest in protein grown 1917 and of sib-pollinated plants from two mixtures of high-protein ears grown 1916. Plants grown in 1918.

Plant number	Percent protein	Plant number	Percent protein
(C23 × B20)–8–3	20.14	(C23 × B20)–6–7	18.54
–8	19.28	–13	18.41
–2	19.11	–6	18.30
–15	19.04	–10	18.18
–7	18.53	–8	17.89
–6	18.47	–3	17.41
–5	18.46	–9	17.28
–12	18.03	–15	16.71
–9	17.94	–5	16.14
–11	17.64	–11	15.63
–13	17.04	–4	15.21
–10	16.88	–1	14.39
–4	15.76	–14	14.18
–14	15.06	–2	13.82
–1	13.87	–12	13.74
Average self...........	17.68		16.39
Parent ear.............	17.59		17.25

Plant number	Percent protein	Plant number	Percent protein
170*–90	17.83	171†–40	18.93
–110	17.24	–90	18.77
–120	17.17	–50	18.36
–140	16.87	–130	17.56
–70	16.55	–120	17.35
–30	16.34	–20	16.82
–10	16.29	–140	16.66
–50	15.77	–60	16.61
–80	15.64	–80	16.43
–60	15.62	–150	15.80
–100	15.82	–30	15.56
–130	14.58	–110	15.05
–40	13.21	–70	14.44
–20	12.79	–10	12.50
		–100	12.46
Average sib-pollinations...	15.84		16.22

* 170 = mixture of three highest ears of 10 selections grown in 1917.

† 171 = mixture of one ear each of the 3 highest of 10 selections grown in 1917.

grown in 1917. The average protein content was 15.84 percent. The second lot, No. 171, was a seed mixture taken from one ear each of the three *highest* of the ten selections of 1917. The average protein content was 16.22. Thus again heredity shows its ruling hand. Selections from the plants having the higher protein contents, produced plants giving the higher protein contents.

Conclusions regarding breeding for high protein

For one acquainted with that vast reservoir of genetic variability—the maize plant—emphasis as to its breeding possibilities has an empty sound. It is sufficient to say that no one knows the limits of progress when breeding to increase or to decrease any one of its characters. What we have to say regarding breeding for high protein, therefore, concerns breeding methods rather than breeding limits.

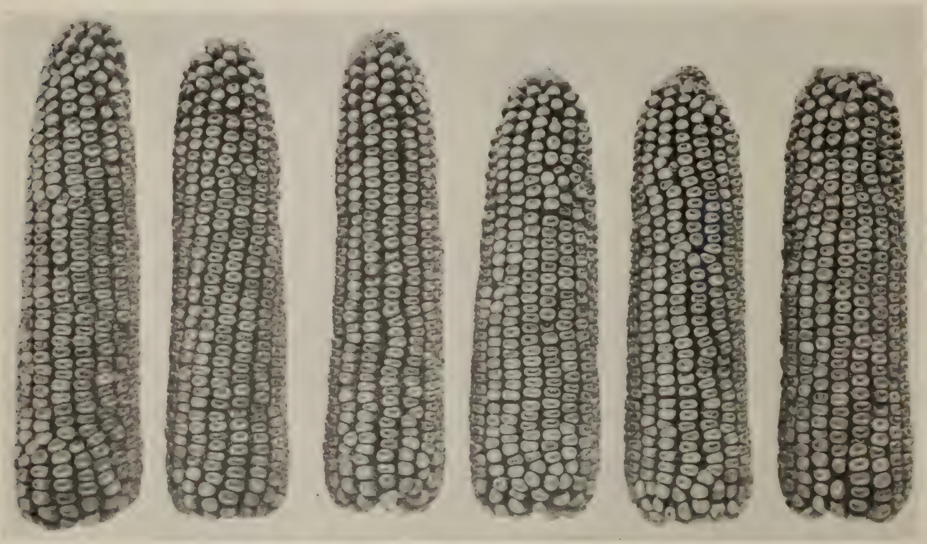

FIGURE 8.—Ears of F_1 hybrid plants (14–30 × 14–6), a cross between two families of Stadtmueller's Leaming selected for high protein. Average protein content, selfed ears, 14.66 percent.

High-protein maize can be secured in the shortest possible time and with a minimum expenditure of effort only when selection is based upon an accurate control of the true biological units and when the germ-plasm contributed by each sex is given due consideration and equal opportunity of expression. In practice the basis of such a method is self-fertilization.

The results obtained from continued selection for high protein in self-fertilized lines depend almost exclusively upon the heredity of the original plants chosen as progenitors. Unless adequate possibilities for recombination are thus present, no amount of selection can create the qualities sought, since there is no evidence of frequent mutation. Obviously the chances for success do not depend wholly upon the number of individuals used, and the rigidity of selection. External conditions must be such as will bring out the highest expression of the desired character, and correlation between personal characteristics and genetic constitution must be fairly high; but given these conditions, progress depends upon the magnitude of the operations at the beginning rather than at the end.

In reality this statement is but a rephrasing of old genetic postulates, and their application to the specific problem of breeding maize for high protein. But what of the result? A high percentage of protein may be produced by this method with certainty and rapidity; yet high percentage composition does not insure high production per unit of area. There is a certain amount of antagonism between high yield and high protein; and even if this were not the case, selection for one character alone would tend to be at the neglect of the other. Merely as a matter of probability it would be more difficult to secure a high proportion of certain ingredients together with high yield than it would be to secure either alone. But the truth is that inbred strains showing the highest percent protein are weak and unproductive. As a rule high-protein strains are less vigorous than strains not so selected, and crosses between them generally give lower yields than other crosses. It may well be, therefore, that high-protein maize can be secured only at the expense of maximum total production. Whether it is worth while to produce special types of maize with increased proportions of certain ingredients in spite of their reduced yields, need not be discussed here.

Such results as are possible can be obtained most easily, we think, by combining strains obtained by self-fertilization and selecting again from the recombinations obtained. Protein content is due to a large number of inherited factors; and various strains having the same percentage composition probably differ in respect to the factors inherited, so that there is a real chance for progress in their union. Since at the same time such a method increases productiveness by means of heterosis it thus serves two purposes.

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GENETICS, NOVEMBER 1920

TABLE OF CONTENTS

PAGE
PAYNE, FERNANDUS, Selection for high and low bristle number in the mutant strain "reduced" 501
EAST, E. M., and JONES, D. F., Genetic studies on the protein content in maize 543

A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS

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Reprinted from GENETICS 6: 311–365. July, 1921

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A Periodical Record of Investigations Bearing on Heredity and Variation

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Entered as second-class matter February 23, 1916 at the post office at Princeton, N. J., under the act of March 3, 1879. Entered at Baltimore, Maryland.

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A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS

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Received February 11, 1921

TABLE OF CONTENTS

INTRODUCTION........ 311
The types used........ 311
The work of the early hybridizers........ 315
Experimental work........ 318
  The parental strains and their behavior........ 318
  Nicotiana rustica humilis crossed with *Nicotiana paniculata*. Experiment 1........ 322
  Nicotiana rustica humilis crossed with *Nicotiana paniculata*. Experiment 2........ 331
  Cross between *Nicotiana rustica scabra* and *N. paniculata*. Experiment 3........ 340
  Cross between *Nicotiana rustica brasilia* and *N. paniculata*. Experiment 4........ 342
  The descendants of the F_2 generation of the plants of experiments 1 and 2........ 342
Summary and interpretation of results........ 349
LITERATURE CITED........ 365

INTRODUCTION

In 1909, I made a cross between *Nicotiana rustica* L. and *Nicotiana paniculata* L., in order to observe the behavior of the progeny of a hybrid in which the individuals of the first generation are only partially fertile. These species were selected because the investigations of several early hybridizers had shown them to be particularly suitable for such a study. The results obtained seem to be of considerable genetic interest, because of the indication from other sources that a high proportion,—perhaps a majority,—of the domestic plants and animals may have been derived from hybrids where the immediate cross is not completely fertile, in a somewhat similar manner. A short abstract of the work was published in 1915, but activities connected with the war have prevented a thorough consideration of the matter until now.

THE TYPES USED

The strains of *N. rustica* L. under observation were received from Dr. O. COMES through the Office of Plant Introduction of the U. S. DEPARTMENT OF AGRICULTURE. They are evidently descendants of some of the plants which he used in compiling his monograph on Nicotiana published in 1899.

N. rustica is well known as a robust annual, becoming biennial or triennial in sub-tropical regions, having cordate, sub-cordate, or ovate hairy leaves and a more or less branched panicle of greenish yellow flowers. Its origin is unknown, as it is said to have been cultivated over the greater part of North America in the time of COLUMBUS. It was the first species of Nicotiana to be taken to Europe, and the first to be cultivated by early Virginia colonists, though soon replaced by varieties of *N. tabacum* obtained from the West Indies and South America.

COMES (1899, p. 20) used practically the same description for the species as DECANDOLLE (Prodromus XIII, I. p. 563). This description, however, appears to be a generalization which will include all of the varieties grouped under the name, rather than a distinct type.

COMES accepts a division of the species into six varieties, of which he gives descriptions and figures; but it must be said that neither his words nor his drawings emphasize adequately the differences between them. The differences are for the most part merely quantitative, it is true, but there are characters which, as extreme deviates, may be said to separate at least four types, *asiatica* to *jamaicensis*, *brasilia*, *texana* to *humilis*, and *scabra*. As a matter of record, I have produced a large number of pure lines from crosses between these types, which, when grown side by side, could easily be distinguished from each other in the field. But, as in most cases where one is dealing with a plant which has been under cultivation for a long period of time, the distinguishing marks bear description only in the case of the extremes.

Whether the varieties actually grown by COMES and sent out under the varietal names noted above, do in truth correspond with the types from which they were originally described, is uncertain. After examining all of the material in the GRAY HERBARIUM and the NATIONAL HERBARIUM, I am of the opinion they do not. But this has little bearing on a genetic investigation. The varieties received under the names *asiatica*, *brasilia*, *humilis*, *texana* and *scabra* were distinct. The variety termed *jamaicensis* was not markedly different from *asiatica*, when grown under optimum conditions; and when grown under poor conditions approached *humilis*. These observations coincide with those of SETCHELL under Californian conditions, although COMES places *jamaicensis* nearer *texana*.

Those interested in the pure taxonomy of the subject should consult COMES's monograph of 1899, and SETCHELL's comments on plants raised from COMES's seed, published in 1912. I will simply describe the four types used in the experiments.

N. rustica var. *texana* (Naud., hort. Paris.) Comes.—Monographie Nicot., 21, t. IX. This variety seems to be the most generalized type of *N. rustica.* It is moderately branched, with comparatively long internodes, growing under different conditions from 1.25 m to 1.75 m high. The leaves are slightly rugose, ovate, and sometimes somewhat cordate. The handsome loose panicle is well filled with flowers. The copy of COMES's detail plate (figure 1) shows the characteristics of the flowers and fruit, although the corolla lobes are hardly as deeply cut or as sharply pointed as represented there. Figure 12, b, is representative.

N. rustica var. *brasilia* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot., 22, t. XI. This variety compares with *texana* externally as does DE VRIES's mutant *gigas* with its progenitor *Oenothera Lamarckiana.* It is a heavy-set plant, a *rustica* with the parts telescoped together and enlarged. The stem is thick and angular from the large vascular bundles which go to the huge leaves. The plant is comparatively short, in typical strains hardly reaching 1 m, but the leaves are large, thick, and very rugose and bullate, showing more of a tendency to a cordate base than any other variety. As a commercial plant, it evidently outyields all other *rustica* varieties, for the broadly ovate leaves are often 50 cm long. The tendency to produce few lateral branches would also seem to be of some advantage in this respect. The stout panicle is so shortened that in the young plant it approaches a head. The flowers like the other parts are short and stout. COMES's detail drawings are corroborated by my own observations. Figure 10, b is characteristic of the young plant, but as the plant becomes older the inflorescence elongates somewhat.

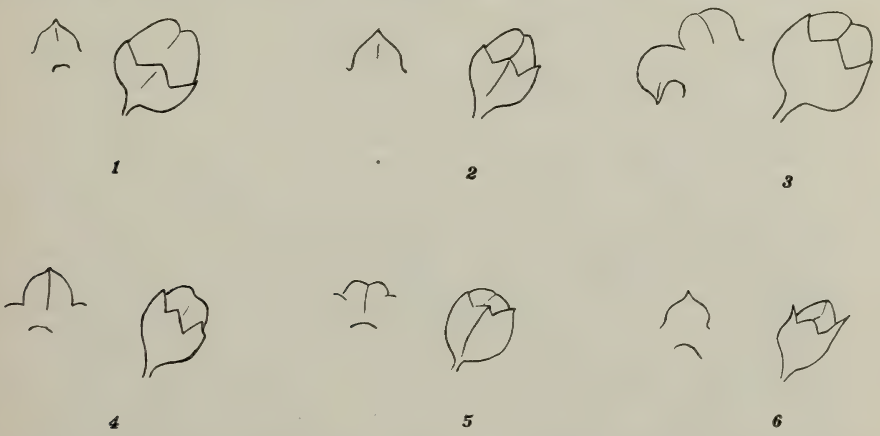

FIGURE 1.—*Nicotiana rustica*, flowers and fruit. (1) *texana*, (2) *jamaicensis*, (3) *brasilia*, (4) *asiatica*, (5) *humilis*, (6) *scabra*. After COMES.

N. rustica var. *humilis* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot. 23, t. XIII. This variety may be said to be a small, robust *texana* of very early maturity. Though it seldom grows to more than 50 cm in height, with leaves in proportion, it is an exceedingly vigorous plant. The general habit of growth is very reminiscent of *texana*, but the leaves, flowers and fruit of a typical plant are easily distinguishable from those of other varieties. The leaves have a rather bright polished appearance, are somewhat fleshy, and tend toward an orbicular shape. The lobes of the corolla and the shape of the fruit also approach rotundity. Figure 2 is from a characteristic specimen.

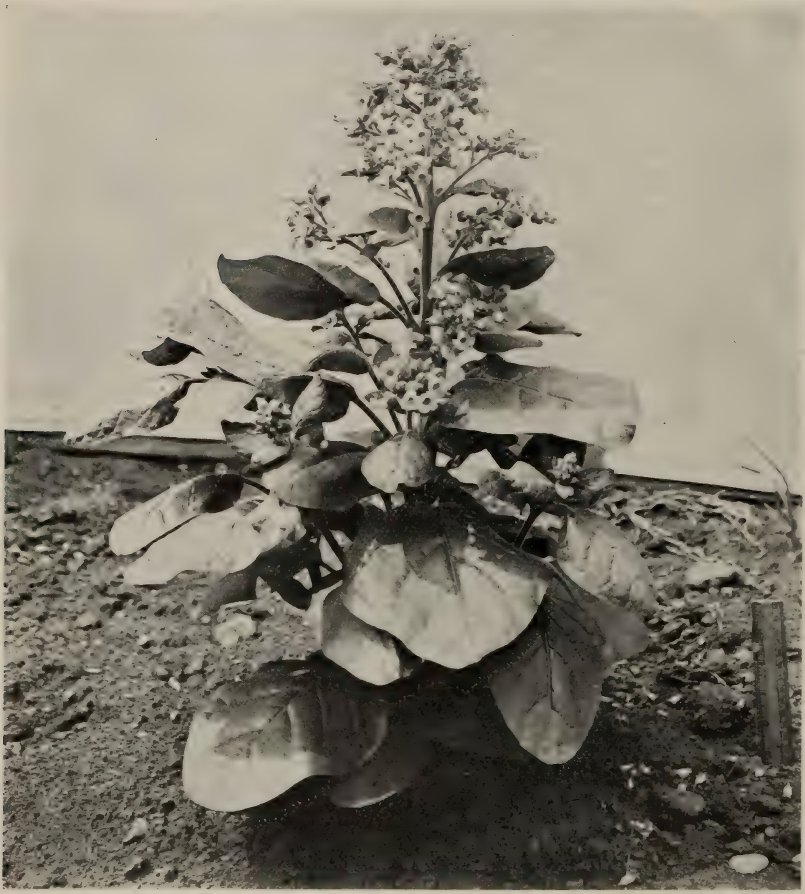

FIGURE 2.—*N. rustica humilis* Schrank.

N. rustica var. *scabra* (Cav.) Comes.—D. C. Prodr. XIII, I, p. 564.—Monographie Nicot. 23, t. XIV. Variety *scabra* may be considered as somewhat the opposite of variety *brasilia*. It is tall, sometimes reaching 2.0 m, and though branching freely, is compact because of the decidedly ascendant tendency of the laterals. Leaves and stem are markedly pilose. As in SETCHELL's specimens,

> "it lacks glands except on the flowering axes, being clothed elsewhere by a thick and compact covering of white slender hairs abruptly bent in the middle. Above, among the flowers these are mixed with the ordinary stalked, multicellular glands commonly found in the species Nicotiana."

The buds and young twigs have a dull purplish color which disappears as they develop. The panicles are loose, and rather delicate. Flowers keep on developing for several weeks even in the axes of mature fruits. The corolla tubes are narrower and the fruits more elliptical than in the other varieties. Figure 11, a, is typical.

The plants of *N. paniculata* L. used in these investigations were obtained from four different scources. One lot came from W. A. SETCHELL, (his number $\frac{10}{07}$): of its orgin I am ignorant. A second lot came from O. COMES. Other collections were made by C. T. BRUES and by W. E. CASTLE in Peru. The species does not seem to be variable, as each of these strains was so similar to the others that they might well be taken for samples of the same pure line.

N. paniculata L.—D. C. Prodr. XIII. I, p. 561. R. & P., Fl. Per., t. 129. This plant hardly needs description. It has been grown in all parts of the world since 1752 when it was discovered in Peru. Figure 3 is from a photograph of a typical plant. It is a low plant branching freely at the base, with cordate pubescent long-petioled leaves, and having long loose panicles of tubular flowers. The flowers are yellow-green, gibbous below the limb. The narrow limb is concave, and slightly fine-lobed. It may be reflexed when in full bloom.

THE WORK OF THE EARLY HYBRIDIZERS

Though one cannot be certain as to the varieties of *N. rustica* used by the early hybridizers, there is no question but that the plants which formed the material for KÖLREUTER, SAGERET, NAUDIN, GODRON, GÄRTNER and FOCKE in their experiments with crosses between *N. rustica* and *N. paniculata*, did actually belong to the two species described above.

The cross *N. rustica* × *N. paniculata* has the honor of being the subject of the first investigation in plant genetics. It was made by KÖLREUTER in 1760, and the first hybrid generation studied the following year. The reciprocal cross was not quite so easy to make, but, after some failures, it was also successfully grown in 1762.

The two species remained favorite subjects for study by the early plant hybridizers. HEDWIG, WIEGMANN, GÄRTNER, NAUDIN, GODRON and FOCKE, made more or less extended experiments with them, crossing and recrossing them in accordance with the methods in vogue before MENDEL'S time. I have examined the papers of KÖLREUTER, WIEGMANN, GÄRTNER, NAUDIN and FOCKE, but will follow roughly the compilation made by FOCKE. The papers of HEDWIG and GODRON I have not been able to obtain.

The hybrids of *N. rustica* × *N. paniculata* were exactly the same as those of the reciprocal cross, although GÄRTNER found the one with *N. rustica* as the mother, to be somewhat more fruitful than the other.

KÖLREUTER and most of the other observers found a high degree of uniformity among the first generation progeny; but GÄRTNER obtained some examples which had shorter and broader flowers than usual, and was able to distinguish several hybrid types. FOCKE'S hybrids, also, were variable, but the reason in his case (and possibly with GÄRTNER) was the use of several varieties of *N. rustica* in their production.

KÖLREUTER states that the hybrid plants were practically intermediate between the two parents in all characters, except that they were partially sterile. GÄRTNER, on the other hand, found them to have considerably more resemblance to *N. paniculata* than to *N. rustica*, especially in their rounded leaves, their stickiness and their long corollas. FOCKE'S observations were just the opposite of GÄRTNER'S. He had great difficulty in distinguishing the hybrids from *N. rustica*, but found little similarity to *N. paniculata* except in the habit of growth and the numerous glands. Measurement of the flowers of FOCKE'S hybrids and their parents were as follows:

	N. rustica	F_1	*N. paniculata*
	mm	mm	mm
Length of corolla	14	19	26
Least diameter of corolla	7	6	5
Greatest diameter of corolla	9	8	6
Spread of corolla	18	15	9

All of the hybridizers found the first-generation hybrids to be very vigorous and of early maturity. FOCKE obtained some dwarf specimens, however, although these blossomed 2 weeks before *N. rustica* and 4 weeks before *N. paniculata* sown at the same time.

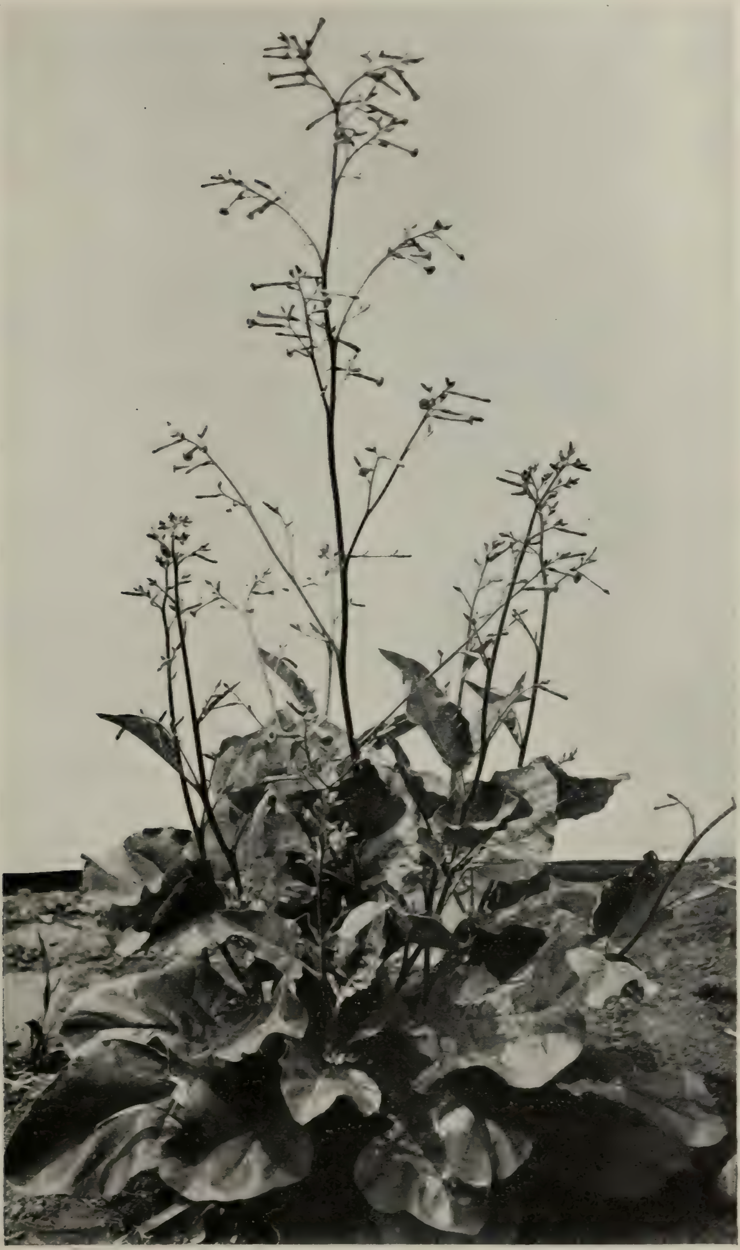

FIGURE 3.—*N. paniculata* L.

Kölreuter concluded that the plants were more fertile with the pollen of either parent than with their own. He even found a higher degree of fertility with the pollen of a variety of *N. tabacum* known as *N. perennis* than when the plants were mated *inter se*. Gärtner's experiments showed the hybrids to be more easily back-crossed with pollen of *N. paniculata* than with that of *N. rustica*.

Focke states that he obtained seed by back-crossing with pollen from *N. Langsdorffii*, but the plants coming from these seeds reproduced the original hybrids. Perhaps he met here a phenomenon that I have observed when attempting to cross Nicotiana species which seldom produce hybrids. Seed is produced which gives again the maternal species. I have attributed the matter to polyembryony effected through the stimulus of the foreign pollen.

The hybridizers all seem to be in agreement that the plants of the second hybrid generation, produced by sib-matings of the partially sterile first hybrid generation, are extremely variable both in morphological characters and in fertility. Kölreuter, Gärtner, Naudin and Focke each found that the second-generation plants resembled *N. rustica* rather than *N. paniculata*, and they were also of the opinion that those plants which stood nearest to *N. rustica* were the most fertile. Wiegmann, on the other hand, observed a similarity to *N. paniculata* in later generations.

Naudin obtained 12 second-generation individuals which differed very materially the one from the other, ranging from 30 cm to 120 cm in height. One plant had lanceolate leaves, something never observed in the parent species. These plants were stated to have been more fertile than those of the first hybrid generation. Plants of the third hybrid generation similar to *N. rustica* were found to be the most fertile.

EXPERIMENTAL WORK

The parental strains and their behavior

The first point of genetic importance to which attention should be called in connection with this material is the uniformity of the strains. Each of the four varieties of *N. rustica* and the strain of *N. paniculata* used in the crosses were selfed for two generations before any quantitative data were recorded. Presumably they had been self-fertilized either naturally or artificially for many generations before, if one may judge from the slight variability exhibited. *N. rustica texana* (348) was indeed somewhat variable in height, ranging from 36 to 75 inches (90 to 190 cm); but in habit of growth, shape and texture of leaves, size of flowers and fruit, it was very uniform. *N. rustica scabra* (352), *N. rustica brasilia* (349), and *N. rustica humilis* (350) were likewise uniform in all characters, even in height. Table 1 shows height measurement on *N. rustica brasilia* and *N. rustica scabra*. The impression of variability given by the measurements is a little misleading, however, since a slight difference in height of the panicles was obliterated in the mind of the observer by the general similarity of the plants in their various characters.

The most uniform variety of *N. rustica* was the small strain known as *humilis*. In one season the range of height measurements on somewhat more than 100 plants was less than 15 cm, and visitors often remarked that they did not know it was possible to obtain a strain of plants so lacking in variability when grown in open field. Similar remarks were made about *N. paniculata* (331). Height measurements on a population of several score of plants usually exhibited a maximum range of less than 12 cm. The plants appeared as if they might have been cut out by a steel die.

TABLE 1

Height of crosses between Nicotiana rustica scabra (352) and N. rustica brasilia (349).

VARIETY OR CROSS	CLASS CENTERS IN INCHES																						TOTAL
	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	
349				4	10	22	14	7															57
352										2	1	5	11	16	17	6							58
352 × 349 F_1													1	3	0	5	5	6	1	1			22
349 × 352 F_1															3	5	2	4	6	5	1	2	28
(349 × 352)-1 F_2				2	3	6	2	7	12	8	12	13	12	12	4	15	4	0	1	0	1		114
(349 × 352) 1-1 F_3	4	9	32	43	17	12	2																119
(349 × 352) 1-2 F_3									3	4	6	10	9	7	28	13	10	8	3				101

Several crosses were made between the *N. rustica* varieties, and their behavior noted through three filial generations. These crosses were *N. rustica scabra* × *N. rustica brasilia*, *N. rustica texana* × *N. rustica brasilia*, *N. rustica scabra* × *N. rustica humilis* and *N. rustica texana* × *N. rustica humilis*. It seems unnecessary to describe this work at length. Three points are to be noted, however, as pertinent to the discussion of the species hybrids described later. (1) The reciprocal hybrids were very similar to each other. (2) The varieties differed from each other by many heritable factors. This is indicated by the greatly increased vigor of the F_1 generations, and by the extreme variability of the F_2 generations. (3) In no case could each of the four *rustica* varieties be reproduced from a single cross.

N. rustica scabra × *N. rustica brasilia*. In table 1, it is shown how much taller the F_1 plants were than the average between the two parents.

The F_1 generation average was even pushed considerably beyond the average of the taller parent (352). The different F_1 generations were very similar to each other in habit of growth, size of leaf, size of flower and size of fruit. Height measurements indicated a slight difference in favor of the cross using *N. rustica brasilia* as the female parent, but the divergence is hardly enough to be significant even in that case.

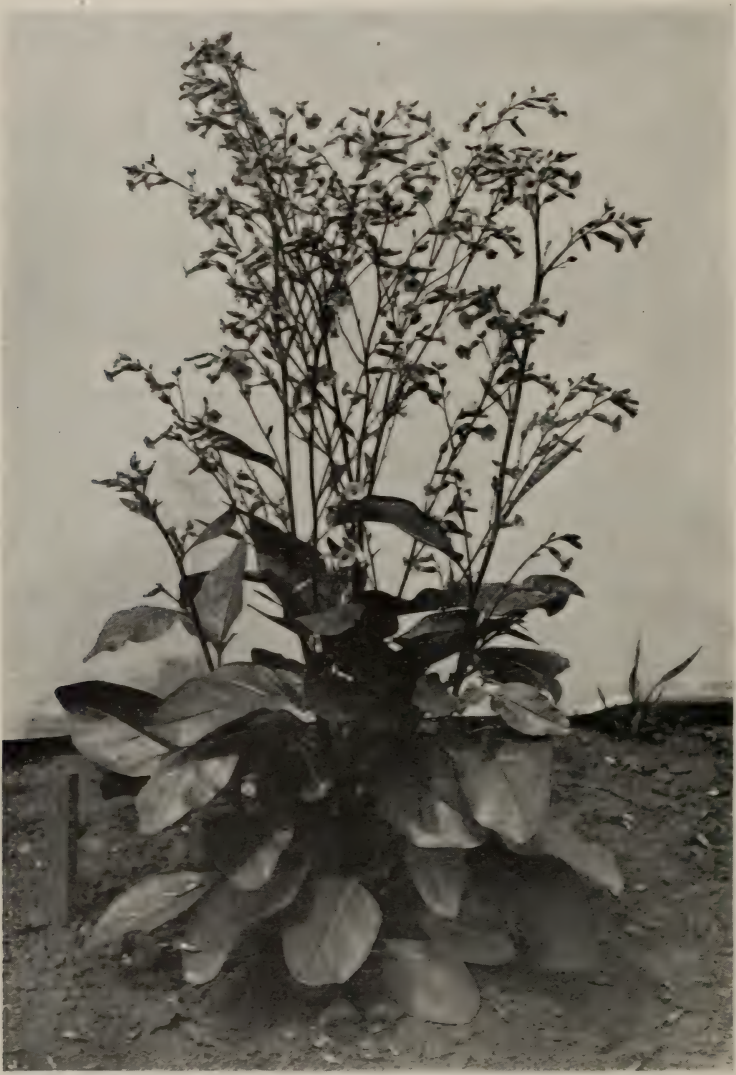

FIGURE 4.—*N. rustica humilis* × *N. paniculata.* F_1 plant.

The variability of one of the F_2 generations is illustrated very well by the height measurements. The possibility of obtaining strains rather uniform in height by selecting extreme F_2 individuals is also manifest.

The most important fact connected with this cross, however, and with the other crosses as well, cannot be demonstrated statistically without covering too much space. It is this: The F_2 individuals, though variable, were what might have been expected by a plant breeder familiar with the two strains used in the cross. They exhibited recombination of the existing characters. Plants similar to *N. rustica texana* were found, but no plants similar to *N. rustica humilis.* Presumably *texana*-like plants arose from the following combinations: Loss of *scabra* hairs; non-appearance of *brasilia* habit of growth, with nevertheless a distinct modification of the *scabra* habit; absence of bullate leaves; and an intermediate size and shape of flowers and fruit, verging toward those of *scabra.* The peculiar smooth fleshy leaves of *N. rustica humilis* did not appear in any members of the F_2 generation.

It might also be mentioned here, that the *gigas*-like habit of *N. rustica brasilia* appeared to be a simple recessive, but it was so changed by numerous modifiers as to make classification difficult.

N. rustica texana × *N. rustica brasilia.* In this cross the same vigorous F_1 generation and variable F_2 generation appeared. No *humilis*-like or *scabra*-like plants were found. Neither the fleshy leaf of the former nor the characteristic hairs of the latter appeared, though certain individuals had small narrow-tubed flowers and oval capsules much resembling *scabra.*

N. rustica scabra × *N. rustica humilis.* The height of the F_1 individuals in this cross, unlike that in the first two crosses, was somewhat less than that of the taller parent. The F_2 was again extremely variable. *Humilis*-like plants were regained, and *texana*-like plants appeared; but no plants resembling *brasilia* were found. The appearance of plants similar to *N. rustica texana* was not unexpected, for given the absence of the *scabra* hairs, the characteristics of *texana* are somewhat intermediate between *scabra* and *humilis.*

N. rustica texana × *N. rustica humilis.* This cross was similar to the preceding one except that the characteristic hairs of *scabra* were absent as well as the *gigas*-like characters of *brasilia.*

The general conclusion from this work is that the four *N. rustica* varieties studied differ the one from the other in a comparatively large number of heritable characters; but that by crossing any two of them, the characteristics of both the others *can not be reproduced.*

Nicotiana rustica humilis crossed with Nicotiana paniculata
Experiment 1

Nicotiana rustica humilis (350) crossed with *N. paniculata* (331) was the hybrid selected for particular study.

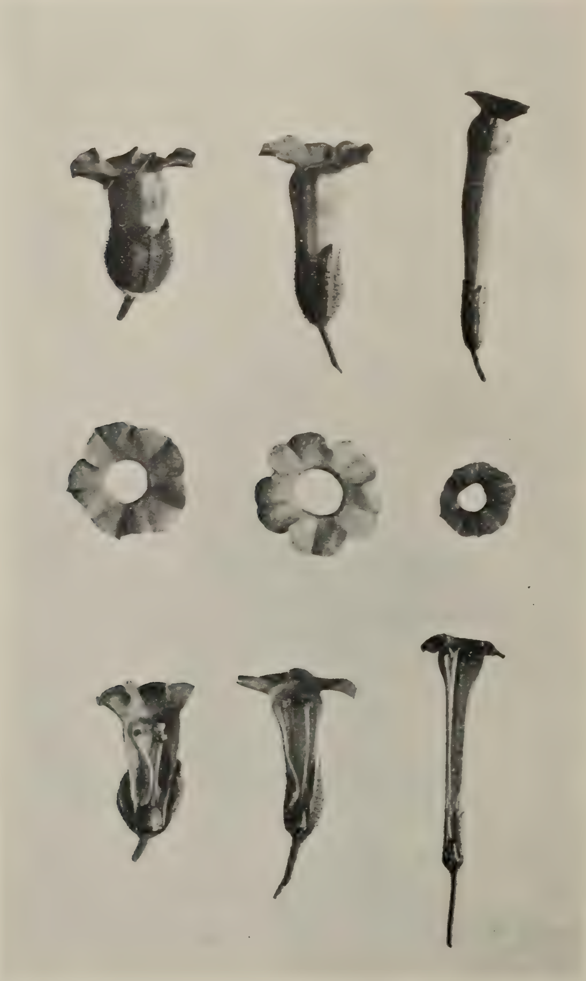

Figure 5.—At left, *N. rustica humilis;* center, *N. rustica humilis* × *N. paniculata* (F_1); at right, *N. paniculata.*

Hybrids between *N. paniculata* and two other varieties of *N. rustica,* viz., *brasilia* and *scabra,* were made successfully, but were not carried beyond the second hybrid generation. The only point to be emphasized now in regard to them is that they were extremely uniform in all characters.

The reciprocal cross with *N. rustica humilis* was also made. This hybrid was not quite so easy to obtain as when the parentage was reversed, but the only difference noticeable in the reciprocal F_1 populations was a slightly taller plant in the case of *N. rustica humilis* ♀ × *N. paniculata* ♂.

My first F_1 population of the cross *N. rustica humilis* (350) × *N. paniculata* (331) was grown in 1910. In leaf size, shape and texture, and in shape of corolla, the plants were very similar to *N. rustica humilis.* In fact, any taxonomist would have classified them as *N. rustica* plants.

But when examined carefully it was seen that they showed the influence of the *N. paniculata* parent, particularly in the intermediate length of corolla and in the spreading habit of growth,—the branches ending in loose *paniculata*-like panicles. These characteristics are probably better shown in figures 4 and 5 than they would be by any amount of description and measurement.

TABLE 2

Variations in length of corolla tube in cross between N. rustica humilis (350) and N. paniculata (331).

MATERIAL	CLASS CENTERS IN CENTIMETERS									
	1.125	1.375	1.625	1.875	2.125	2.375	2.625	2.875	3.125	3.375
350, plant 1 (1910)............		24	1							
350, plant 2 (1910)............	1	23	1							
350, 5 flowers each (1910)......	33	168	63							
331, plant 1 (1910)............						2	22	3		
331, plant 1 (1910)............							24	1		
331, 5 flowers each (1910)......						47	178	40		
(350 × 331) F_1, plant 1 (1910)..				24	1					
(350 × 331) F_1, 5 flowers each (1910)......................		2	9	208	63					
(350 × 331) F_2, 1 flower each (1911).................	2	7	31	90	76	24	7	3	1	1

I have already spoken of the uniformity of the two parental types in all characters. The F_1 plants were just as uniform in their traits, though they were about 25 percent taller than *Nicotiana paniculata* (the taller parent). Table 2 gives an illustration of this remarkable lack of variability.

The F_1 plants produced only from 1 to 30 seeds per capsule when crossed *inter se* or when self-pollinated, even though considerable quantities of pollen were applied. This sterility seemed to be equally a sterility of ovules and of pollen for no greater quantities of seed were produced when pollen of the F_1 plants was applied to either parent, or when the pollen of either parent was applied to them.

Matings 331 × (350 × 331) F_1 and 350 × (350 × 331) F_1 were somewhat difficult to obtain, but after a score or so of attempts to use F_1 pollen on each of the two pure species, several capsules averaging 10 seeds each resulted. The reciprocal crosses, i.e., the use of the pollen of the pure strains upon the flowers of the F_1 plants, were usually successful, the capsules ranging from 3 to 22 seeds each. Perhaps the very fact that back-crosses with the F_1 pollen were harder to obtain than the reciprocals, shows that ovule sterility was less than pollen sterility. But when they matured at all, the back-crossed capsules of each of the four combinations possible contained about the same quantity of seed. Consequently it seems reasonable to attribute this apparent functional difference between the ovules and the pollen of F_1 plants, to the greater protection against inclement conditions received by the ovules, rather than to inherently greater fertility.

The plants of both (350 × 331) × 331 and (350 × 331) × 350 showed the general uniformity of the true F_1 generation, though the former were considerably more variable. The back-cross with *N. paniculata* (331) took on somewhat more of the *paniculata* characters, and that with *N. rustica* (350) resembled *rustica* more than did the F_1 generation. For example, the flowers of the *paniculata* back-cross averaged about 2.3 cm by 65 cm in length and breadth of the corolla tube, while those of the *rustica* back-cross averaged 1.9 cm by 0.9 cm.

By exercising great care in pollinating two F_1 plants *inter se*, a quantity of seed was obtained from which 246 plants were raised in 1911. The percent of germination of the seed is not known.

The F_2 plants were each unlike all the others though in general they resembled *N. rustica* more than *N. paniculata*. In height they varied from 24 cm to 170 cm. Flowers from 21 plants are shown in figure 6 compared with flowers of the two grandparents in the upper right-hand and lower left-hand corners respectively. It is clear that all sorts of combinations between the long narrow corolla of *N. paniculata* and the short broad corolla of *N. rustica* are in evidence, but the point of particular interest is that the extremes exceeded those of each parent. The same point is emphasized by figure 7, in which the largest leaves of a number of F_2 plants are shown on the same scale.

A good many measurements of the various characters of this F_2 generation were made, but reports of series of measurements on particular characters serve to confuse rather than to clarify the points which seem important.

For this reason a number of photographs of F_2 plants are submitted in place of detailed measurements.

Though nearly all of the plants of this generation would have been grouped as *rustica* plants, there were seven which resembled *paniculata* rather closely. Of these, four retained traces of *rustica* descent. The other three plants duplicated *N. paniculata* exactly. Figure 8 shows one of these segregates. By comparing it with figure 3, a representative of the pure species, one can see that it is no exaggeration to use the word exactly. In every feature of habit of growth, of leaf and of flower, these plants were wholly *N. paniculata.*

Naturally the suspicion arose that these plants were *paniculata* individuals which had been included by mistake, or had arisen as volunteers. Nevertheless there was some evidence this was not the case. In the first place, the seeds from which the plants were grown, were planted in sterilized soil, and the seedlings transplanted very carefully and tagged in the field. Some misplacement was found, of course, for no one can carry on extended experiments without error. But on examining the field of some 10,000 plants, the traceable misplacement was found to be about 1 plant per 2000, while here were three plants in a lot of 246 for which to account. In the second place, though two of these *paniculata*-like plants apparently were as fertile as the pure-line *paniculatas*, *one was completely sterile.* The fact that two of these supposed segregates were so fertile is admittedly a somewhat suspicious circumstance, but the appearance of a sterile plant duplicating *paniculata* characters seemed to clinch the claim of that particular individual as a segregate from the original cross.

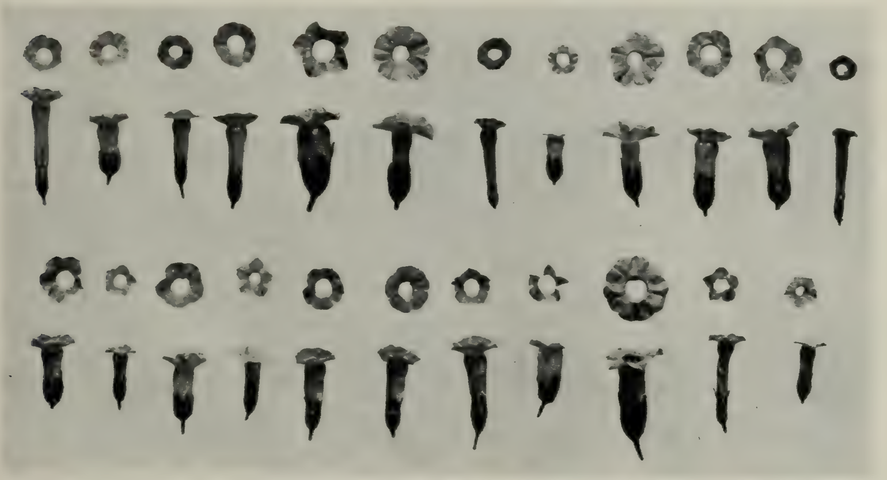

FIGURE 6.—Flowers from 21 plants of F_2 generation *N. rustica humilis* × *N. paniculata.* Upper right, *N. paniculata;* lower left, *N. rustica humilis.*

Seed from self-fertilized flowers of one of the fertile plants was grown the next year, and the progeny bred true to the *paniculata* characters.

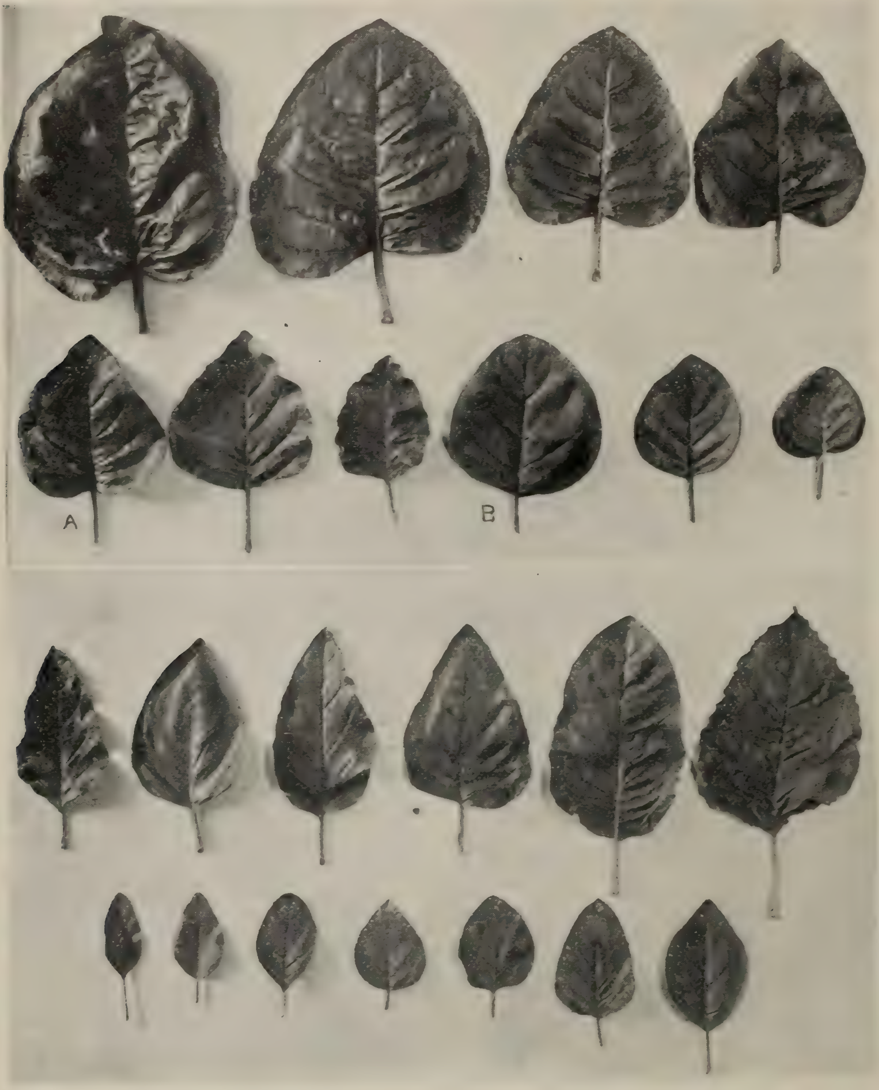

FIGURE 7.—The largest leaf on each of the 21 plants of F_2 generation *N. rustica humilis* × *N. paniculata* of which the flowers are shown in figure 6. A, *N. paniculata;* B, *N. rustica humilis.*

Not a trace of influence of *N. rustica* appeared. This fact combined with the others mentioned made me so apprehensive of the whole matter that the experiment was repeated in its entirety. This second investigation of the *rustica-paniculata* cross is described later in the paper. Suffice it to say now, that I am convinced from it that the three *paniculata*-like plants of the F_2 generation under discussion were in fact segregates.

Several reasons for accepting this view will appear later. We will mention here only the reason why the complete fertility of the supposed segregate need not be taken as a particularly suspicious circumstance. It is this: Though the F_1 plants were very slightly fertile, their fertility was uniform. The F_2 plants, on the other hand, showed as great a variability in the fertility exhibited as in any other character complex. Several plants could not be induced to set a single seed in the limited number of trials made, either with their own pollen, with pollen of any of their sibs, or with pollen from either of the species entering into the cross. In others, there were varying degrees of fertility up to complete fertility as far as the quotas of seed set are concerned, though some small percentage of pollen abortion was found in the best.

Among the plants of the F_2 generation, 6 or 7 were found which reproduced the characters of *N. rustica humilis*, one of which is illustrated in figure 9. None of them was markedly sterile, though varying degrees of fertility were exhibited. But the fact that *N. rustica humilis* was reproduced as a segregate need not astonish one. The astonishing fact was that, though variety *humilis* entered into the cross, and though variety *humilis* crossed with other varieties of *N. rustica* did not throw segregates duplicating varieties *brasilia* and *scabra*, nevertheless *these varieties were duplicated* in the segregates when *humilis* was crossed with *paniculata*.

Figure 10 shows an F_2 segregate of this population at the left, compared with a *brasilia* plant at the right. Though the photographs do not emphasize the points of similarity, in the field they were very marked. This plant could only have been classified as a *brasilia* plant, though the leaves were not so bullate as in the particular pure-line *brasilia* plant with which we have chosen to compare it. On the other hand, several other pure lines of *brasilia* plants have been grown which do not exhibit such a bullate appearance as the one here shown; and further, the particular way the sunlight fell on this plant while the photograph was being taken, rather obscures the amount of leaf-wrinkling it possessed.

In figure 11 a typical plant of variety *scabra* is shown at the left, while a *scabra*-like segregate appears at the right. In this picture, one who is familiar with the appearance of *scabra* will be struck with the fact that the segregate seems to be more strikingly *scabra*-like than the plant from the pure line. This is because of the fact that the photograph of the true *scabra* plant is so sharply focussed that the grayish appearance due to the

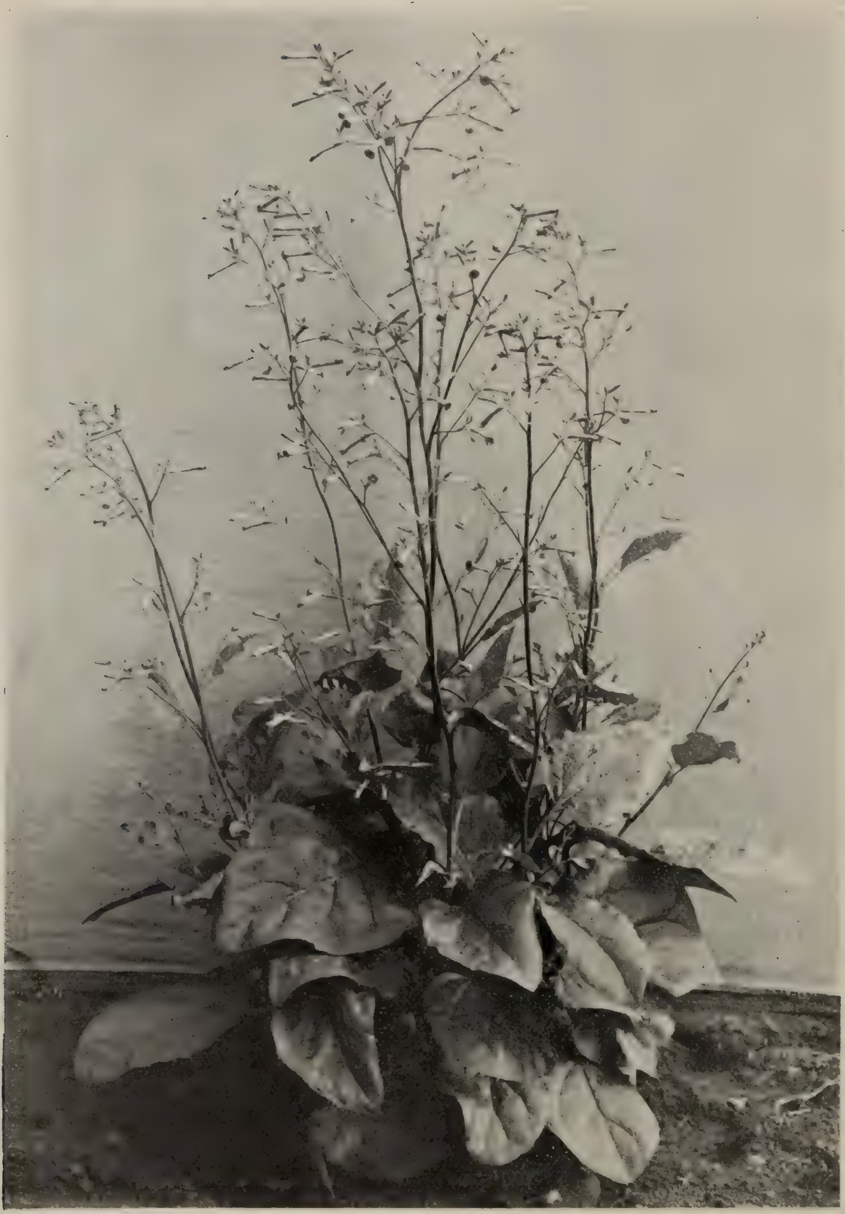

FIGURE 8.—F_2 segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. paniculata*.

hairs is partly obliterated. The habits of growth may seem somewhat different but this is not really true. The segregate is a little more vigorous than the *scabra* plant, and though the photographs were taken when the plants were of the same age, the lateral branches of the *scabra* plant had not developed to the same extent. In another week the pictures would have been practically identical, for the two plants tallied character by character even to the characteristic dull purple in the young flower buds.

Again, we have a third segregate in the left-hand plant of figure 12 which might have been die-cut as a representative of variety *texana* (the right-hand plant). The similarity of the two plants is obvious even in the photographs taken under different weather conditions. Notice specially the size and shape of the flowers, the method of branching, and the character of the panicles. The leaves are the same shape, size and texture, and are set at the same angle on the stalks.

A few other extreme plants are interesting in this connection. A rather gigantic form is pictured in figure 13. It is not extremely tall, as can be seen by comparing it with the foot-rule, but the leaves are far larger than any we have examined in the larger cultivated types of *N. rustica*. The largest leaf measured possessed a blade 75 cm × 60 cm. Contrast this huge plant with the little one shown in figure 14, where the whole plant is only 25 cm high and has a maximum leaf size of 9 cm. Oddly enough the size of the flowers is not noticeably correlated with the size of the plant as a whole. The flowers of the lilliputian of figure 14 are almost as large as those of the giant sister of figure 13. Another peculiar type is illustrated in figure 15. In texture and shape of leaf, this plant is much like the *brasilia* variety; but the inflorescence is unique. The lateral branches combine with the main stem to produce the appearance of one large corymb. In marked contrast to this type is one shown in figure 16 where the prominently white-veined leaves curl at the edges and the inflorescence is such that the plant has a pyramidal appearance. Again in figure 17, a plant is shown which is quite different from any described variety of *N. rustica*. The sparse leaves are lanceolate in shape and very delicate in texture; the flowers are long and narrow; and the general character of the panicle is much looser than in the other types described, although approached by *N. rustica scabra*.

The photographs of the few F_2 individuals shown in the figures, represent some of the extreme types. Now in imagination, picture graded series of intermediates between these particular plants—hardly two of similar appearance—and you have a fair idea of the F_2 generation of this cross.

It is useless to try to show frequency distributions of particular characters. They fail to serve the purpose, for it is the *combination of characters* which makes every plant distinct from the other. At the same time the measurements of height of plant, length and breadth of leaves, size of flower and of fruit, show clearly four facts which are important for the theoretical discussion which is to be given later. (1) In an F_2 series of less than 300 plants, nearly every individual *seems* to have an hereditary constitution different from all others. (2) In height of plant and in size and shape of leaves, the range is far beyond that of either grandparent; but in size and shape of flower, though combinations of the grandparental

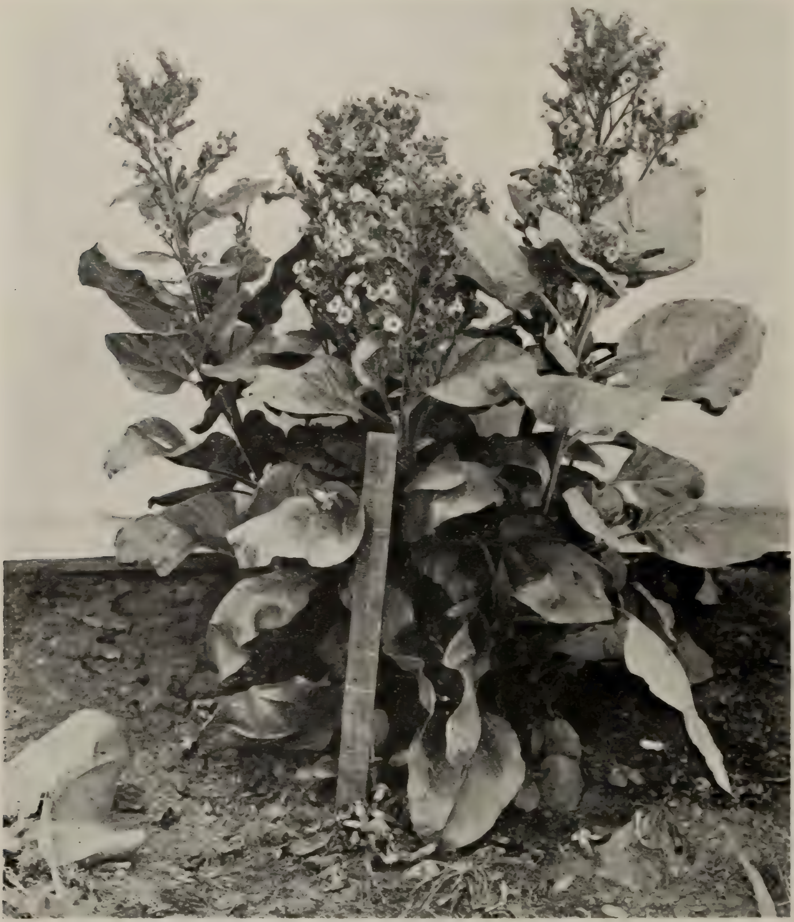

FIGURE 9.—F_2 segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica humilis.*

characters are found, they do not pass far beyond those actually existent in the pure species. (3) Nearly all of the plants resemble *N. rustica*. Those which resemble *N. paniculata* at all, are almost identical with it. In other words, a series of combinations presumably possible, appears to have been omitted. (4) The fertility of the plants varies from a condition where almost all of the pollen is abortive and few if any seeds are produced (limited number of trials) with sib pollen or with pollen of either of the grandparental species, to a condition where every ovule (full capsules) seems to function with "own" pollen (though from 20 percent to 30 percent of this pollen is abortive).

Nicotiana rustica humilis crossed with Nicotiana paniculata
Experiment 2

Owing partly to the suspicion that the *paniculata*-like segregates in the F_2 population of the cross just described might have been "volunteers," and partly to the desire to make some observations that had been omitted, a repetition of the cross between *N. rustica humilis* (350) and *N. paniculata* (331) was made.

The F_1 generation was practically identical with that described under experiment 1. The plants were slightly taller and the flowers slightly larger, but this difference may be attributed to the rich soil upon which they were grown.

Some considerable time was spent studying the fertility of this generation, with the following results. From 1 percent to 7 percent of the ovules were proved to be functional, by careful pollinations with "own" pollen, with sib pollen and with pollen of the two parental species. This ratio was determined by comparing the number of seeds actually produced with the number found in full capsules of *N. paniculata*, as a determination of the number of ovules in a flower of the plants under consideration was difficult. There is some inaccuracy in these figures, therefore, but it is probably not very great. The variation found is due, in part, to manipulation difficulties and in part to the small samples involved; and one may take it that under the best conditions 3 or 4 percent of the ovules of the F_1 generation on an average will produce seeds, and that these will germinate in from 35 to 55 percent of the cases.

Samples of the pollen of F_1, taken from mature anthers, likewise showed a variable ratio of grains apparently perfect. The variation here was from less than 3 percent to about 10 percent, and seems to be due largely to the great difficulty of obtaining random samples. When pollen from anthers

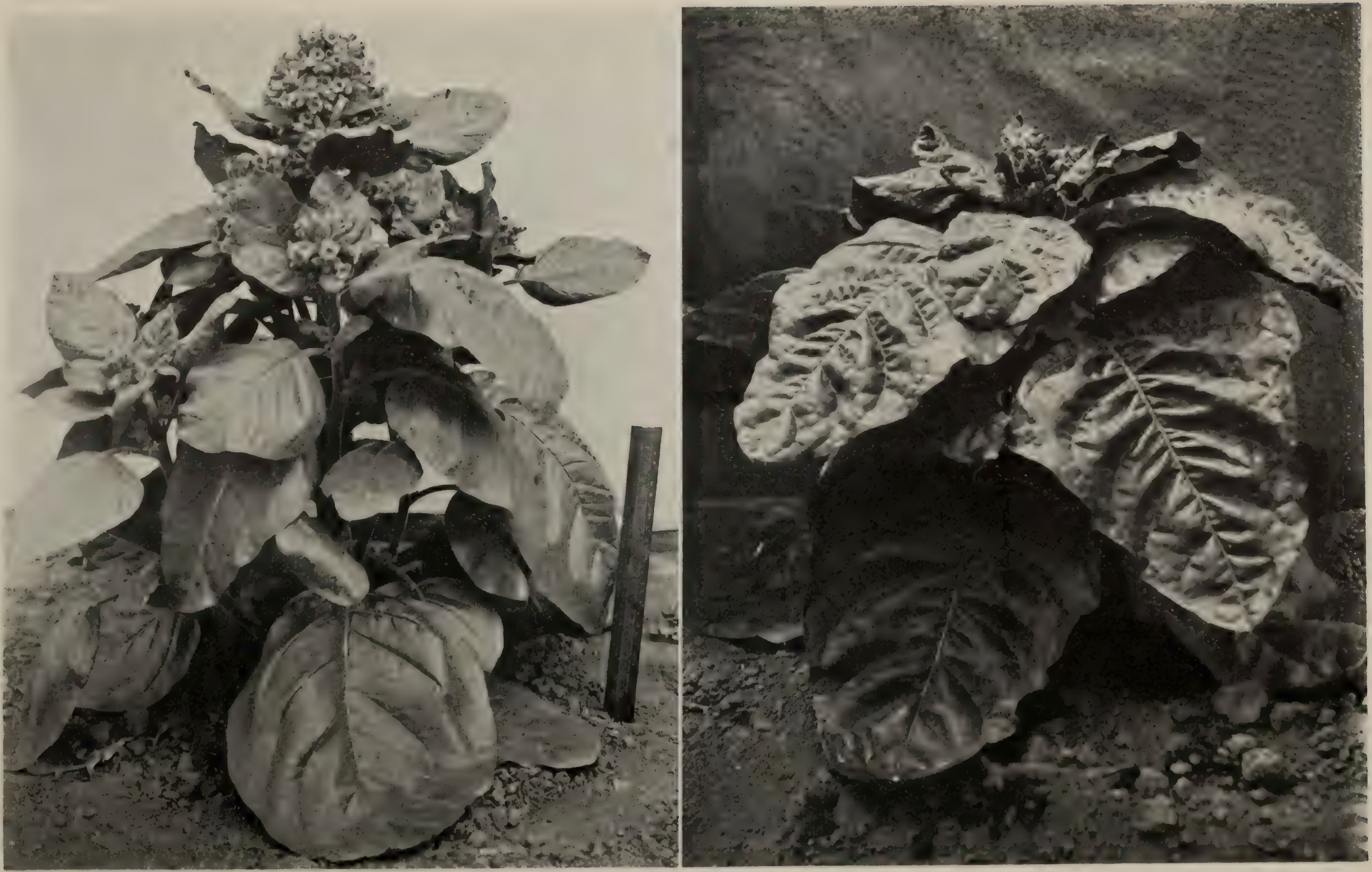

FIGURE 10.—At right, *N. rustica brasilia;* at left, F_2 segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica brasilia* with the exception of the bullate leaves.

just opened is shaken out, the count is distorted because the perfect grains shake out more easily. When the pollen is scraped out with a scalpel the results are a little more uniform, but even with this method they are not to be relied upon. Paraffine sections of mature unopened anthers gave a lower ratio—from 1 to 3 percent—of grains perfect in appearance; but this method also has its drawbacks. Sections from anthers of different phases of maturity, indicate that *many of the pollen grains which appear to be functional in the young unopened anther, dry up as soon as the latter opens.* Furthermore, germination tests made in stigmatic fluid and in sugar solutions, showed that from 20 to 70 percent of the grains which appeared to be perfect would not extrude pollen tubes. It may be argued that such a test does not preclude the possibility of a high percentage of germination of the "good" grains on the plant stigmas. This is true. But experience with the pollen of *N. rustica humilis* and *N. paniculata* in hanging-drop cultures, leads me to believe that over 80 percent germination can be obtained by this means, if the pollen is really functional.

The general conclusion from observations on the pollen of the F_1 generation of this cross, is that less than one-tenth of one percent of the tetrads which the pollen mother cells *start* to form, ever succeed in functioning as pollen.

One other set of observations should be mentioned in this connection, though the conclusions may be revised after more extended investigation. Examination of slides of *N. rustica humilis*, of *N. paniculata*, and of the F_1, indicates that each species has 24 chromosomes as the gametic number, and that a high percentage of breakdown occurs at the heterotypic division of the pollen mother cells.

Of the F_2 generation, 300 plants were raised to maturity. These plants were studied rather carefully, and it is hoped that some day the data may be punched on cards and all of the statistical relations made out with a Hollerith machine. For the present purpose too voluminous citation of these details regarding separate characters would simply result in obscuring the important points.

The plants were again extremely variable. For example, the height frequencies in decimeter classes, for the 288 plants which had reached their maximum height at the time of measurement, were as follows:

Class centers in dm	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Frequency	1	2	1	2	7	10	11	13	19	35	39	35	31	29	17	15	13	2	3	2	1

When one realizes that nearly all of the F_1 plants are included in *one* of these decimeter classes,—less than 10 percent falling into contiguous classes,—this range of the F_2 generation from 3 decimeters to 23 decimeters is tremendous. A similar variation occurred in habit of growth and time of maturity (range of 30 days), in shape, color, leafiness and hairiness of stem, in size, shape, thickness, bullation and hairiness of leaves, and in character of inflorescence (from the head-like panicle characteristic of variety *brasilia* to a loose panicle resembling a raceme). The fruit also showed extreme variation, ranging from a pumpkin shape, one and one-half times as broad as long, to an ovoid type nearly twice as long as broad, and from a length of 3 mm to a length of 18 mm. It is probable, however, that variation in fertility distorted these records. In flower shape and size, variation was not so marked. A few plants had *rustica*-shaped flowers longer than those characteristic of *paniculata* and a few had *rustica*-shaped flowers about one-half the size of those of *N. rustica humilis*; but only one plant approached *N. paniculata* flower characters.

The characters of the plants considered together showed that many of the plants could be classified as resembling varieties *humilis*, *brasilia*, *scabra*, and *texana* as in experiment 1. There were others which resembled those figured previously, as well as a few new types. Only one plant resembled *paniculata* at all closely, though certain features possessed by many individuals, length of corolla, hairiness, cordate leaves, etc., may have been hereditary contributions from this source. The one *paniculata* segregate, curiously enough, was only about two-thirds the size of a normal *paniculata*, though all of the size relations appeared to be the same,—that is, leaves and flowers were smaller in their proper ratios. The plant was 66 cm in height; the leaves were the exact shape, texture and hairiness of *paniculata*; the flowers also were exactly what one might imagine to be characteristic of a dwarf *paniculata*,—19 mm long and 3 mm broad, flaring at the end to 6 mm. About 20 percent of the pollen grains appeared to be normal, but the many attempts at self-pollination were all failures.

The appearance of this plant in this population, is to some extent a confirmation of the results of experiment 1. It gives more confidence in the supposition that the three plants apparently identical with *N. paniculata* found in that F_2 population, were in truth segregates from the F_1 generation.

From the standpoint of genetic theory, the questions connected with the fertility of this family seemed important, and a serious effort was made to discover the facts. Since the inquiries were subject to the limitations already described, the effort was not all that might be desired; nevertheless, I believe that the data have a decided value, and that the conclusions drawn from them are satisfactory from the standpoint of statistical theory.

First, the pollen from 52 plants was examined microscopically. Representatives from all of the more distinctive types were included. The pollen was examined dry, in water and in glycerin. The general results are as follow:

(1) Judging from the amount of pollen formed and from the percentage of aborted grains, it appears uncertain that any F_2 plants are more sterile than the plants of the F_1 generation. In nine plants out of 52 examined, the proportion of pollen formed and the proportion of grains apparently viable were comparable with the proportions found in F_1. The grains apparently viable were between 3 percent and 10 percent of the total.

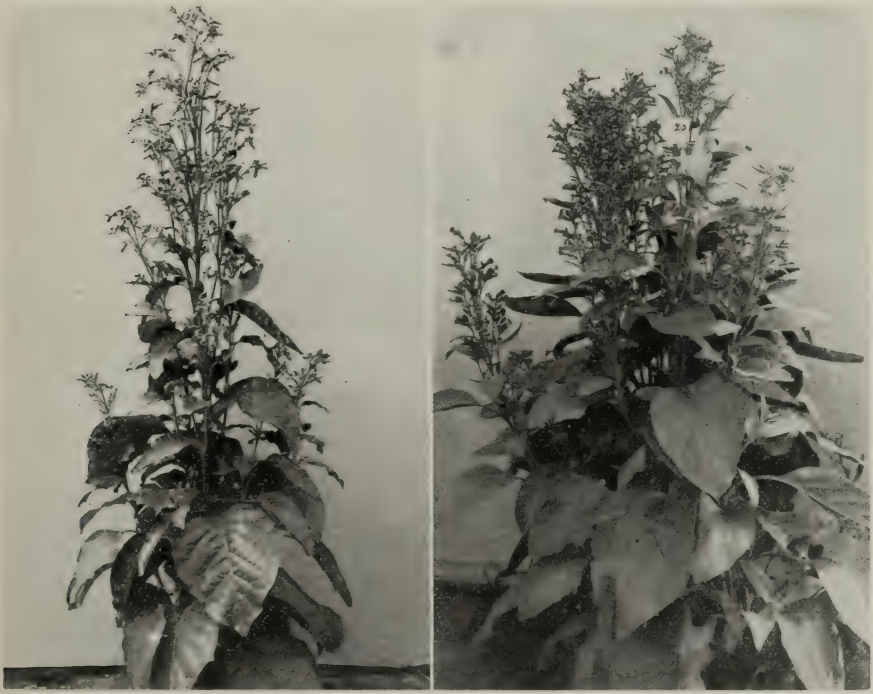

FIGURE 11.—At left, *N. rustica scabra;* at right, F_2 segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica scabra.*

In one other plant, only aborted pollen grains were found, but it is assumed that this condition was not due wholly to heredity because the plant formed about 25 percent of the number of seeds expected normally when pollinated with sib pollen.

(2) Ninety percent of the plants produced much greater complements of good pollen than those of the F_1 generation, and the percentage of grains apparently viable ranged from 10 percent to 80 percent. No plants in this generation produced a proportion of "good" pollen equal to that of the pure species (95 percent to 98 percent), but three plants of the F_2 generation described in experiment 1 had over 96 percent "good" pollen. The modal condition was around 65 percent "good" pollen.

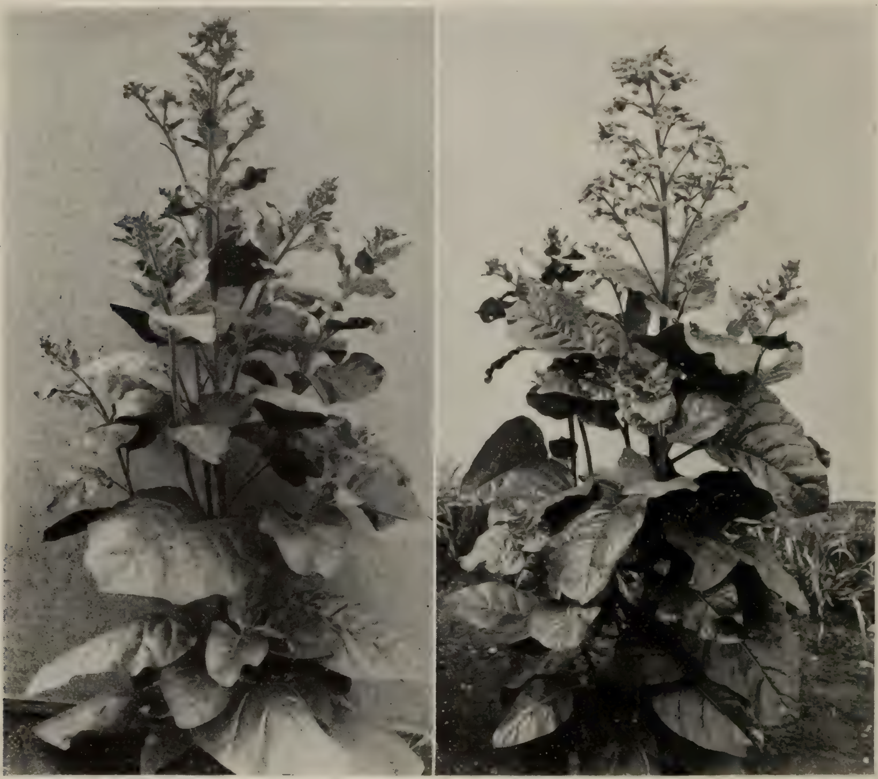

FIGURE 12.—At right, *N. rustica texana;* at left, F_2 segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica texana.*

(3) No decided correlation between plant characters and fertility as determined by this method could be discovered.

(4) No pollen germination tests were made on this population, either in stigmatic fluid or in sugar solutions, but judging from the complements of seed formed naturally (probably sib-pollination), many of the "good" pollen grains did not function. This conclusion was drawn from the fact that no seed was obtained on several plants producing some apparently

"good" pollen. It might be concluded that this result came about because of a greater proportion of sterility among the female gametes, but the general evidence is against such a conclusion.

The shortcomings of the pollen-examination method of estimating fertility are obvious. Because of them another method was used. It has its faults, but I believe that by its use a reasonably accurate comparison of the fertility of the F_1 and F_2 generations can be made, and this is the essential point.

Each plant was examined carefully at the stage when the seeds are just beginning to turn brown. The flowers had had a fairly equal chance of being pollinated naturally with sib pollen; and the seeds formed had matured sufficiently to give a fair idea of those retaining some measure of vitality, since abortive seeds had begun to shrivel. The procedure was as follows. The percentage of capsules formed per plant having been given weight in the judgment, several capsules from each individual were carefully "peeled" and an estimate of the fertility made from the seeds therein. The plants were thrown into eleven fertility classes ranging from 0 to 10. It may be assumed that classes 5 to 6 represent about a half complement of seeds, class 10 represents complete fertility on the female side, and class 0 represents complete sterility. The results of an examination of 269 plants are given in table 3.

TABLE 3

Estimated fertility of the F_2 generation (350 × 331) through an examination of the capsules.

Class	0	1	2	3	4	5	6	7	8	9	10
Frequency	4	4	11	23	34	20	41	32	56	28	17

The curve is not as smooth as it would have been, had not the records been taken in classes numbered 1, 1½, 2, 2½, etc. The tendency was to enter the records in the integral classes. But this fact does not interfere with the main result. On this scale the plants of F_1 belong uniformly in class 2. In other words, the F_2 plants were variable in fertility, but 97 percent of them showed greater fertility than those of the F_1 generation. At least 12 percent of the plants gave full complements of seed which seemed perfect in every way.

On the other hand, one *can not* conclude that a single plant—through heredity—was less fertile than the F_1 generation, *though such may be the case.* Let us look at the matter a little more carefully. Suppose it be admitted that the F_1 plants showed a slightly greater fertility than class 2. This would leave 19 plants out of 269 F_2 plants apparently less fertile than those of the F_1 generation. At the same time it must be remembered that the F_1 plants were pollinated very carefully. A great deal of pollen was applied, and hundreds of flowers were tested. A similarly extensive test on each of these F_2 plants was impossible. Each plant self-pollinated (162) had only 5 flowers manipulated (with the exception of the *paniculata*-like plant), taking the usual precautions, though the capsules pollinated naturally were examined carefully. To the best of our knowledge and belief, therefore, evidence for a fertility less than that of F_1 exists for only the 8 plants of the first two classes, *and this evidence is not conclusive*, except possibly in the above-mentioned *paniculata*-like individual. In the first place, each of these plants might have produced *some* seed if pollinated as carefully and extensively as were our F_1 plants, since each produced as great a percentage of apparently viable pollen as the average F_1 plant. In the second place, in any family of plants individuals appear occasionally that are completely sterile for reasons which seem to be unconnected with heredity. Some of these plants may be of this type. It seems to me, therefore, that the following conclusion is in accordance with all the facts. *Ninety-seven percent of the F_2 plants produced in the cross between N. rustica humilis and N. paniculata were more fertile than the F_1 plants, and it is not certain that the remaining 3 percent were less fertile.*

One hundred sixty-two of these F_2 plants were self-pollinated. In almost every case 5 flowers were used. Unfortunately the prevalence of aphis and an excess of rain and wind during the remainder of the season ruined a very large part of this work. Only 94 plants matured seed, and of this number only 70 produced 75 or more seeds. Seed was collected from 203 plants where natural pollination had taken place, however, and the germination of each lot tested. One hundred seeds from each plant were counted, placed in separate Petri dishes on moist blotting paper, and kept in a rather warm greenhouse. The results were rather interesting. While most of the seeds—particularly the seeds of those plants which showed a high percentage of germination—sprouted after 4 or 5 days, a number of the dishes showed a continuous germination week by week,—several seeds germinating after 63 days. It would have been decidedly worth while to have traced the life history of some of these plants germinating at different periods to see if there were marked differences in their characters, but this was impossible.

Table 4 is a correlation table made up from the germination tests of 67 plants from which both selfed seed and open-pollinated seed were obtained. To those who know how uniform germination tests made from duplicate samples of seed usually are, it may seem strange that the correlation between the two sets was only 0.33 ± 0.07. But this is not really so odd. The conditions under which the selfed seed matured were bad, but not uniformly bad. Some bags had aphides under them, others were free from aphides; some were wet on the inside, others stood up well and were only wet on the outside. Nevertheless, the results here and in table 6, show that many of these plants were in a rather balanced condition as regards maturing seed. Little changes in environment evidently affected the maturing capsules much more than similar changes would have affected pure lines, since several pure lines under observation at the same time gave germination tests of over 90 percent. Thus there is pretty good evidence for supposing that zygotes were eliminated with a high degree of frequency by external conditions; and it seems to me that it is more than a guess to say that there was a selective elimination of plants with particular hereditary complexes.

TABLE 4

Correlation in percent of germination between open-pollinated and self-pollinated seed produced by F_2 plants of cross between N. rustica humilis and N. paniculata.

	Percent open-pollinated									
Percent self-pollinated	5	15	25	35	45	55	65	75	85	
5	1	3	1		2	1	1	1		10
15	2	2	2	3	1	2	2			14
25		1		3	4					8
35	1	1	1		3					6
45		2		1	2		2			7
55		1	1		1	2	1			6
65	1	1	1	1			2			6
75		1			2		3	1	3	10
	5	12	6	8	15	5	11	2	3	67

$r = 0.33 \pm 0.07$

TABLE 5

Percent of germination of open-pollinated seed produced by plants of the F_2 generation of cross between N. rustica humilis and N. paniculata.

Percent germination	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
Frequency	16	17	14	17	19	12	13	15	17	9	13	10	9	11	5	1	3	2

Table 5 gives the percent of germination of the open-pollinated seed.

Cross between Nicotiana rustica scabra and N. paniculata. Experiment 3

Before continuing to describe the descendants of the F_2 generation of the cross between *N. rustica humilis* and *N. paniculata* (experiment 2) two crosses between *N. paniculata* and other varieties of *N. rustica* should be discussed for the sake of comparison. The first of these is one in which *N. rustica scabra* was used as the female.

Both when the plants are considered as units and when specific characters are examined, the F_1 population compares in uniformity favorably with those already described. For example the height frequencies of 49 plants in decimeter classes starting with 7.5 are 6, 15, 19 and 9. Again the flower-length frequencies in millimeter classes beginning with 19 are 4, 20, 22 and 3.

The fecundity of the plants seemed to be slightly greater than in the other crosses, in that a larger percentage of self-pollinated flowers produced some seeds. But since the number of seeds per capsule obtained was about the same, it is not certain that the gross fertility exhibited was any greater.

The 257 F_2 plants raised to maturity showed again an extreme variability, ranging from 30 cm to 210 cm in height, and reproducing *brasilia*, *scabra*, *texana*, *asiatica*, *humilis*, *nana* and *gigas* types as before.

Three differences between the plants of this cross and those of the cross into which variety *humilis* entered were found, however, and these differences appear to be important. (1) *All* of the plants leaned decidedly toward the *rustica* side of the house. Not a single plant was found that one could say truthfully was *paniculata*-like, although traces of possible *paniculata* influence could be seen in fully half of the population. (2) Small plants with a starved appearance were found in considerable numbers, (38 out of 257 plants). In the cross between *N. rustica humilis* and *N. paniculata* scarcely two plants were of the same type, yet here 15 percent of the population were dwarfs that were fairly uniform in appearance. These plants resembled the one reproduced in figure 14, except that few seed capsules were formed. Flowers pollinated carefully by hand, as well as flowers pollinated by insects, fell off after 3 or 4 days. Toward the end of the season a few capsules were formed, but they contained no normal seed. A few collapsed seeds in which no embryo could be discovered, were all I obtained for my trouble. Pollen from these plants examined in glycerin appeared to have as high as 3 percent of normal grains, but there was no evidence that this appearance was a true criterion of vitality. *A part of these plants was tested carefully, and I believe it safe to say that they were less fertile than those of the F_1 generation.* (3) Among the other plants of the population a varying degree of fertility was found, with the tendency toward high fertility as in experiment 2; but none showed decided evidence of being less fertile than the plants of the F_1 generation. Thus there was a high correlation between a physical type (the sickly dwarf), and sterility. Perhaps the phenomenon met here is due to a lethal factor similar to those described by MORGAN for *Drosophila melanogoster;* at least one may suppose it is not the same type of absolute sterility found to be questionable in the F_2 generation of experiment 2. This experiment was carried no further.

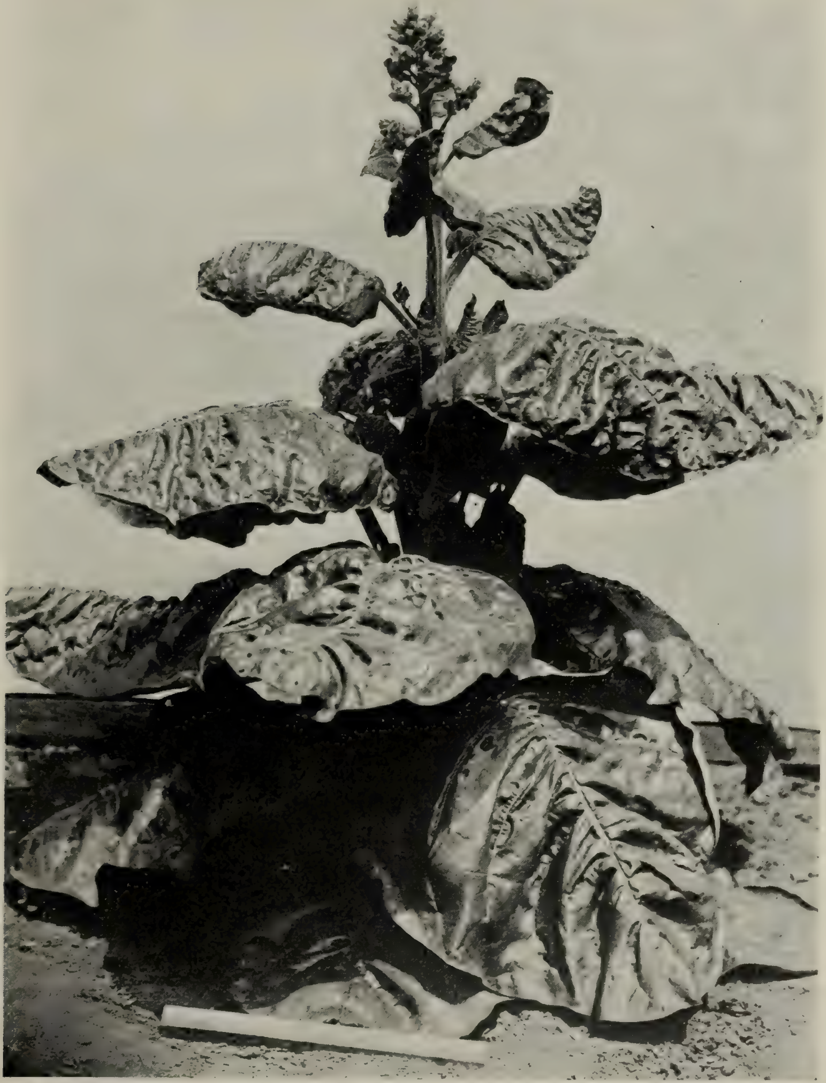

FIGURE 13.—Giant F_2 segregate *N. rustica humilis* × *N. paniculata.*

Cross between Nicotiana rustica brasilia and N. paniculata. Experiment 4

The cross in which *N. rustica brasilia* entered as the maternal parent, was very similar to that in which variety *humilis* was used as the *rustica* parent. Time for studying this cross in detail was not available, but 26 plants of the F_1 generation and 57 plants of the F_2 generation were grown and examined. The uniformity in physical appearance and in fertility characteristic of the other F_1 generations was again found here. Similarly also the F_2 generation was extraordinarily variable, and produced individuals resembling *brasilia, humilis, texana, scabra* and "*nana.*" Nothing can be said concerning the fertility of the plants except that it was variable. Judging from open-pollinated capsules, the majority of the plants were more than 50 percent fertile, but whether any plants were less fertile than those of the F_1 generation is unknown.

The descendants of the F_2 generation of the plants of experiments 1 and 2

A considerable number of F_2 plants from experiments 1 and 2, *N. rustica humilis* × *N. paniculata*, were selfed, and the descendants followed for several generations. The behavior of the families of each experiment was so similar that there is no advantage in describing more than one set. In table 6, therefore, the salient features of 51 F_3 families arising from F_2 plants of experiment 2 are set forth.

The important question to be answered by these data is that of variability, and it was not an easy matter to make an estimate which would be quantitative and at the same time practical. Fifty-one families were raised, giving me a real task in trying to cover the point at issue without covering an interminable number of pages. If, for example, frequency distributions were made of a number of characters in each family, there would be an apparent accuracy in the results. But this accuracy would in truth be only apparent. What we wish to know is the variability of the families as entities. With series of data for separate characters, it would be necessary to try to keep the figures for each character in mind, and to weight these figures mentally in making a general average. Instead of doing this on paper after taking the data, therefore, it was done in the field. For this reason the figures of table 6 may be subjected to the criticism that they are distorted by the personal equation of the writer. This is true. But, on the other hand, all figures are somewhat distorted by personal equations, and I believe the data of table 6 give a better idea of the actual situation than would have been given by a more voluminous set.

The first column in the table gives the number of the F_3 family. In the second column is the number of plants under observation. When the plants showed a distinct type, it is listed by name in the third column. Under height, leaves, flowers and fertility, the measurements of the extremes are given in the first column. In the second column under each heading a number is given which represents the variability of the character in question on a scale of 10 where 1 represents the wonderful uniformity of the pure line of *N. paniculata* described previously and 10 represents the inordinate variability of the F_2 generation of experiment 2. Finally column 4 gives the general variability of each family on the same scale. This figure is frankly an estimate. But it was made in the field after the estimates had been made of the variability in height, in size and shape of leaf, in size and shape of flower and in fertility. It gives weight to these determinations, but it also considers the general characteristics of the plants in a manner that could only have been done by an observer in the field.

Owing to the small number of plants grown, families 52, 101 and 176 should be omitted. Four other families, 67, 199, 228 and 234, may also be omitted because it was so difficult to estimate their variability on a scale comparable to the others. Family 199 contained 48 plants. All but 5 of these plants were typical *N. rustica humilis* plants with a variability scarcely greater than that of the maternal great grandparent. The 5 aberrant individuals were tall plants resembling *N. rustica texana*. Omitting the *texana*-like plants the general variability of the family would stand at 2, but its rating with them included is questionable. Similar remarks might be made for the other three families. Perhaps it is better to rest our conclusions on the 44 families remaining after the omission of these seven.

Two facts stand out impressively in this array of data:

(1) The families are all rather high in fertility and low in the variation in fertility within each group. Several families showed scarcely any discernible variation in fertility and No. 131 should probably be ranked the equal of its great-grandparents. Every ovule was capable of being fertilized, and the amount of abortive pollen (3 to 5 percent) was so small one would hardly suspect the origin of the strain. (2) The uniformity of the families, visualized as relative homozygosity of each parental F_2 individual, is much greater than one would expect. Twenty-two families, just one-half of the total, were rated 3 or less in general variability. When it is recalled that *N. paniculata* our No. 1 in the scale is the most uniform strain ever raised in our experimental garden, whether of Nicotiana or of

TABLE 6

Characteristics of the F_3 families of cross between N. rustica humilis and N. paniculata.

F_3 FAMILY NUMBER	NUMBER OF PLANTS	TYPE	GENERAL VARIABILITY	HEIGHT		LEAVES		FLOWERS		FERTILITY		NOTES
				Range	Variability	Range	Variability	Range	Variability	Range	Variability	
24	16	sh. *tex.*	3	80–100	3	15×12–23×22	3	21×16–22×18	2	8–9	2	1 type only
30	20	var.	6	50–110	7	9× 5–30×21	8	15×15–18×15	3	6–9	5	
33	51	*scabra*	2	110–135	2	20×15–24×18	2	18×16–19×17	2	8–9	2	1 type only
49	49	*texana*	4	80–150	5	20×12–32×23	4	17×15–19×16	3	9–10	2	1 *bras.* type
52	7	var.	6	70–110	5	20×14–23×16	3	20×13–21×19	5	7–9	4	
56	34	*hum.-tex.*	5	50–160	7	12×10–24×22	6	19×14–22×19	3	6–8	5	
64	30	sh. *tex.*	3	100–140	3	14× 8–25×16	3	20×16–22×15	2	5–8	4	1 type only
67	27	*scab.-tex.*	4	110–150	3	15× 9–25×22	5	18×15–19×15	2	8–9	3	4 plants 60 cm.
76	37	*texana*	4	70–160	6	13× 8–23×20	4	18×16–23×20	5	7–8	4	Few lvs.
79	38	Var.	6	70–150	6	15×10–25×19	6	18×13–22×18	5	7–9	3	lvs. obl.-lanc.
83	51	*humilis*	2	100–120	3	18×13–20×16	2	18×16–19×17	2	9–10	2	lvs. thick
85	51	*scabra ?*	2−	130–170	2	23×16–25×18	1	18×18–19×19	1	8	1	late maturing
87	50	*hum.-tex.*	4	60–120	3	15×10–28×22	4	17×13–19×19	4	7–8	2	
88	35	*texana*	4	90–160	4	20×15–32×28	5	18×17–19×18	2	8–9	2	lvs. *bras.*
89	28	sparsely lvd.	2	105–130	2	25×20–30×22	2	18×15–21×20	3	7–8	2	
90	25	new type	2	100–140	2	25×20–31×28	3	16×15–18×17	2	7–8	2	habit uniform 1
93	25	*hum.-tex.*	3+	80–120	4	20×12–28×24	4	16×14–20×18	3	7–8	2	
98	30	var. *tex.*	5	60–180	6	20×12–30×26	5	19×18–20×18	2	7–8	3	
101	3	tall *tex.*		130		26×22		18×15–18×16		8		
102	57	*texana*	4	80–150	4	15×10–28×22	5	18×14–20×18	4	7–8	3	
104	56	new type	6+	100–150	5	20×20–30×12	6	17×16–20×19	3	7–8	3	like No. 90
108	33	new type	4	50–150	5	20×12–28×24	4	18×17–21×20	4	7–8	2	tall laterals
110	21	*texana*	2	120–160	2	25×18–28×23	2	18×16–19×16	2	7–8	2	tall type
111	50	tall *bras.*	1+	100–120	1	24×19–28×26	2	18×15–18×16	1	8	1	late
114	44	*texana*	3	60–160	4	15×10–32×25	4	18×16–19×16	2	6–7	3	
115	52	new type	2	110–140	1	25×20	1	18×18–19×18	2	7–8	2	few lvs.

TABLE 6 (Continued)

122	36	*texana*	5	60–150	6	17×10–30×22	4	16×12–20×16	5	8–9	3	
124	52	tall *bras.*	2	120–150	2	18×14–30×19	2	18×18–19×18	1	7–8	3	
131	40	tall *bras.*	1+	100–115	1	25×20–28×26	1	19×16	1	9	1	like No. 111
139	50	*texana*	5	70–120	4	14×10–30×25	5	17×13–19×16	4	5–7	4	
145	50	*scab.-tex.*	5+	60–190	6	15× 8–30×24	6	18×17–19×17	2	8–9	2	very large caps.
146	35	*humilis*	3	50–90	3	15× 8–26×18	3	18×15–19×16	3	7–9	3	
149	52	tall weak	3	130–160	3	28×26–34×29	4	16×15–17×16	3	4–9	5	
150	104	tall *bras.*	2−	110–150	2	25×16–27×18	1	18×14–19×15	1	8–9	1	
159	53	*texana*	3	100–160	3	25×18–28×18	3	19×15–21×16	3	8–9	3	
163	54	sparse *tex.*	2	120–180	3	24×20–26×22	3	20×16–21×17	2	7–8	2	
166	47	*bras.-tex.*	4	90–130	3	26×20–28×24	3	18×16–19×17	3	5–8	4	
167	44	*texana*	5	35–140	6	8× 6–30×26	6	14×12–20×18	6	8–9	2	small type
168	25	elong. *hum.*	5	50–120	6	20×18–28×24	5	18×15–19×15	4	8–9	3	
176	11	6 tall *bras.*	4	110–130	3	18×16–26×22	4	18×15–19×16	4	7–9	3	5 *texana*
191	46	var.	5	40–150	7	15× 8–28×21	6	16×14–21×20	6	8–9	2	
192	43	var.	6	60–160	6	16×15–28×26	7	16×16–18×16	6	8–9	3	also lvs. 28×14
199	48	*humilis*	2?	60–100	2	12× 8–20×15	2	18×15–19×18	3	7–8	4	5 *texana* excluded
213	59	*texana*	5	70–190	6	17× 8–34×26	6	18×13–20×18	3	7–8	3	
214	47	var. *hum.*	4	50–130	5	10× 6–25×20	5	18×18–20×18	3	7–8	4	
217	50	var.	5	80–130	5	16×12–25×20	5	16×15–17×15	4	7–8	3	lvs. sparse
219	43	new type	3	50–110	5	15×13–25×25	3	16×16–18×17	2	8–9	2	lvs. round
228	45	*tex.-scab.*	3	110–180	3	28×20–32×25	2	18×15–20×16	2	8–9	2	3 with small lvs.
234	42	*tex.-scab.*	2+	160–180	1	20×16–28×22	2	18×16–19×16	2	8–9	2	also 2 small pl.
248	38	*texana*	3	100–180	5	18×16–30×26	3	19×17–21×19	3	6–8	3	
250	25	*humilis*	3	60–120	4	16× 8–20×16	3	16×15–17×15	2	8–9	2	

any other genus, the fact is extremely suggestive. Our varieties *texana*, *jamaicensis* and *asiatica* raised from stock self-fertilized for several years and presumably inbred for a long period of time, would have to be rated nearly 3 in variability.

Fifty percent of the F_2 plants of this cross, therefore, were so homozygous as to produce populations comparing favorably with those found in the so-called pure varieties of N. rustica which had been collected from various sources.

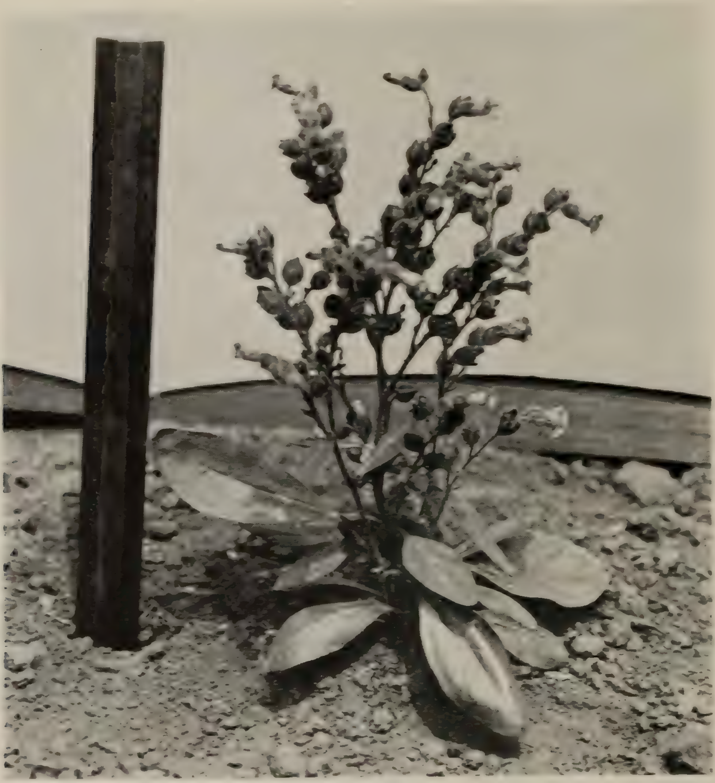

FIGURE 14.—Very small F_2 segregate *N. rustica humilis* × *N. paniculata*.

Now we hope there will be no misunderstanding of the position taken here. It is not maintained that each of these 22 families was as uniform as the "pure" varieties of *N. rustica* they most resembled. But it is believed that none exceeded the "pure" strains by more than one-half point on our scale of 10. In other words both the eye and the measuring-stick told us that the variability of the "pure" strains and the segregate strains was of the same general order of magnitude.

No descendants of the 51 F_3 families of experiment 2 were carried further, but from the F_3 families of experiment 1, which, it will be recalled, were in every respect similar to those of experiment 2, several strains were carried through the F_4, F_5 and F_6 generations,—selfed seed being used in each instance. There were eight families in the F_4 generation, and several of these were split up in the F_5 and F_6 generations. This series of strains does not include the progeny of one of the *paniculata*-like individuals of the F_2 generation, which as we have already stated, was kept under observation. This plant produced a population resembling pure *N. paniculata* in every detail. The family showed no trace of inheritance from *N. rustica*, and was as uniform as a population of the *paniculata* great-grandparent. The individual plants seeded freely. The capsules were *full* of seed and various samples of the pollen showed it to be from 96 to 100 percent perfect, morphologically.

The remaining strains clearly belonged to the *rustica* group. No systematist would have suspected that they were the descendants of segregates arising from a cross where *N. paniculata* was used as one of the parents. Six of the strains were similar to certain *N. rustica* varieties obtained from Dr. COMES. One was clearly *N. rustica humilis*, a second was *N. rustica asiatica*, and a third was *N. rustica brasilia* in appearance. Two other strains were unlike any plants of *N. rustica* we have seen, but nevertheless would have been classified as members of that species. The first of these was a giant form, figure 13 giving a good idea of its characteristics. The second was a small type in which the plants all resembled figure 14. The remaining strains were from variable F_3 families resembling combinations of COMES's varieties.

These strains were grown in order to try to answer two questions which are important in any theoretical interpretation of the results from the experiments considered as a whole. The first question concerns the variability of the families. The second question pertains to the fertility of the individuals.

The behavior of the F_3 families demonstrated conclusively that a much greater number of the F_2 plants were homozygous in the majority of their characters than one would have any right to expect had there been such recombination of hereditary factors as is usually found in varietal crosses, with no selective elimination of gametes and of zygotes. It is important then to know whether this apparent homozygosis is in truth a homozygosis of all the hereditary factors possessed by the individual, or whether recombination with the ultimate attainment of a greater degree of homozygosis is possible. Selfed individuals from the variable populations of F_3, gave populations more uniform than those from which they came. This is probably what should have been expected. Again, two of the selfed populations from F_3 generations rating 2 in general variability, produced populations in which leaf size in one case and habit of growth (this means somewhat more than mere height) in the second case were distinctly less variable than in the parental population. In no other instance could one

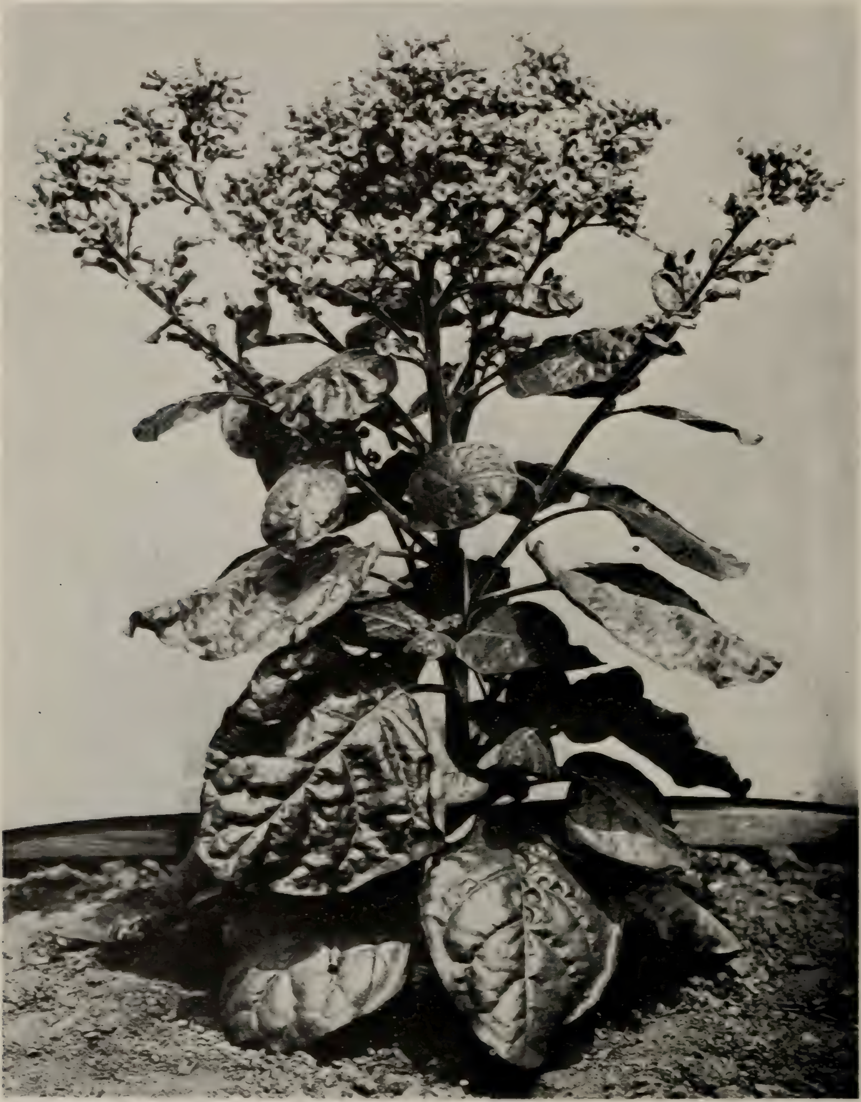

FIGURE 15.—F_2 segregate *N. rustica humilis* × *N. paniculata* showing habit of growth not found in either grandparent.

be certain that the 5 near-homozygous F_3 families tested had produced populations less variable than themselves, but it must be remembered how uniform these families were in the F_3 generation. Conversely, in no instance was a variability greater than that of the parental population observed.

In the case of fertility, not only was the variability of the parental population never exceeded by the progeny, but no individual of a population resulting from the selfing of a particular plant was ever less fertile than its parent. The succeeding generations always *increased* in actual fertility and in uniformity in the production of functional gametes and zygotes. In this series, only the two *paniculata*-like plants of F_2, already described, seemed to have perfect gamete-producing mechanisms, although by the word perfect it is not meant to exclude the possibility of an occasional aborted pollen grain. In the remainder of the F_2 plants, and in their descendants, many plants were found in which the ovules were all functional, but it is probable that none reached the percentage of perfect pollen grains characteristic of pure *N. paniculata* and pure *N. rustica.* In these two pure species, individuals have been found where 100 percent of the pollen grains seemed to be morphologically perfect, but in general there was from 2 percent to 3 percent of abortive grains. Whether this low percentage of aborted grains is due wholly to environment or not I am unable to say. I suspect this to be true, however, because I have found at least that high a percentage of aborted pollen grains in some individuals of every "pure" species of plant I have ever examined. The most fertile plants among the descendants of this cross, excluding the two *paniculata*-like segregates, had about 4 percent of the pollen abortive, with the one further exception of a *brasilia*-like plant where about 2 percent of the pollen was imperfect. Thus while many plants which came out of this cross compare favorably in a general way with the fertility of the two pure species that entered into it, adequate statistical proof that they equaled the pure species in fertility is not available. I believe the general experience with the cross leads one to expect the ultimate production of individuals wholly as fertile as the pure races, but definite proof has not been forthcoming.

SUMMARY AND INTERPRETATION OF RESULTS

Those who have borne with us thus far no doubt will have been impressed with the fact that a very large amount of quantitative data has been more or less roughly lumped together in a descriptive manner. The danger in such a procedure is fully realized. It makes it difficult for the reader to make up his mind as to whether the separate conclusions drawn are really established by adequate and critical data, or whether they are merely tentative. At the same time it must be clear to every one that the essential facts are rather broad and general in their nature, and could have been presented in no other way. I have dealt with the variability of the plants considered as units, rather than the variability of separate characters. In arriving at decisions, it was necessary to deal with characters separately, of course; but in weighting these data on the separate characters in order to accomplish the main objective,—an estimate of general variability,—personal equation necessarily enters. I believe the results justify the procedure, however, for two reasons. In the first place, if each plant character had been studied with all the refinements of modern statistical methods, it still would have been impossible to have judged the plants as units by an impersonal objective summation of these data. The subjective element would have been masked in a quantity of detail, but it would have entered into the final result. Furthermore, I believe that in such work as this the investigator who lives with his plants in the field, who uses all the quantitative data at his command, but who nevertheless brings to his aid all the somewhat intangible facts that intimate experience gives him, is able to come to a better realization of the truth than one who works over cold data obtained by others.

Our position in the matter having been made clear, let us endeavor to pick out the established facts which appear to be essential from the standpoint of genetic theory.

The two species, *N. rustica* and *N. paniculata*, species giving extremely uniform progeny when selfed, have produced reciprocal first-hybrid-generation populations almost identical with each other, and as uniform in all their characters as the parental populations themselves.

The proportion of fertile ovules formed by each F_1 plant was about the same; that is to say, careful manipulation would result in an average seed complement of about 3 to 4 percent of the normal. Each plant seemed to have this figure as a possibility. One might obtain no seed or any proportion of seed up to 8 percent after a pollination, but the variation was as great on one plant as another, and this variation may be taken largely as a mark of failure to provide the best conditions for fertilization plus the variation always existent in random sampling. At least one-half of these seeds will germinate.

An investigation of F_1 pollen formation leads me to believe that less than 0.1 percent of the possibilities of the pollen mother cells in the formation of pollen grains is ever realized, yet a study of mature anthers only indicates that 2 to 3 percent of the pollen grains found there are functional. The histological results on pollen show that one is often seriously misled if conclusions as to what has happened during gamete formation are drawn from a casual examination of the contents of mature anthers. The pollen-germination studies showed further that apparent morphological perfection in a pollen grain is not to be construed as positive evidence of ability to function.

Taken at their face value the data on gamete formation seem to indicate that the formation of female gametes is some 40 times greater proportionately than the formation of male gametes. This result, however, can not be taken to mean that 40 functional female gametes per thousand and only 1 functional male gamete per thousand may be formed because of incompatible hereditary elements entering into the remaining non-functional gametes which do not come to maturity or do not function at maturity. A great part of this differential proportion in the formation of male and female gametes may be due wholly to factors of environment. In other words, the greater size of the cells, the larger amount of cytoplasm, or in general the better mechanism for gamete production in the ovary, make slight changes in temperature, osmotic pressure, etc., less effective in stopping gamete formation in the ovary than in the anther. There is considerable evidence for the truth of this statement in the fact that comparable percentages of seed were produced in each of the following cases, self-pollination, sib-pollination, use of pollen of either pure parental species on the flowers of the F_1 generation, and use of the pollen of the F_1 generation on the flowers of either parent.

The characters of the F_1 plants rather blended those of the two parents, but the resemblance to *N. rustica* was greater than to *N. paniculata*. The plants produced by back-crossing F_1 with each of the two parents, partook of more of the characteristics of the parent used. The variability of the back-crosses was considerably greater, statistically speaking, than that of the true F_1 generation, but botanically the plants were like true F_1 generations in that they were of one general type with much less variability than the F_2 generation in all characters.

The F_2 generation was inordinately variable. With the possible exception of certain flower relations, variation in all characters transgressed those of the grandparents markedly.

Numerous character combinations, predictable with random mating and no selective elimination of gametes or zygotes, were omitted. This statement is made after very careful consideration and a full realization of the theoretical implications. Combinations approaching *N. rustica* were much more frequent than those approaching *N. paniculata*. It is not largely a question of salient characters being masked or of group heredity (i.e., linkage), for the characters have been considered separately as well as in groups. *N. paniculata* differs from *N. rustica* in numerous characters.

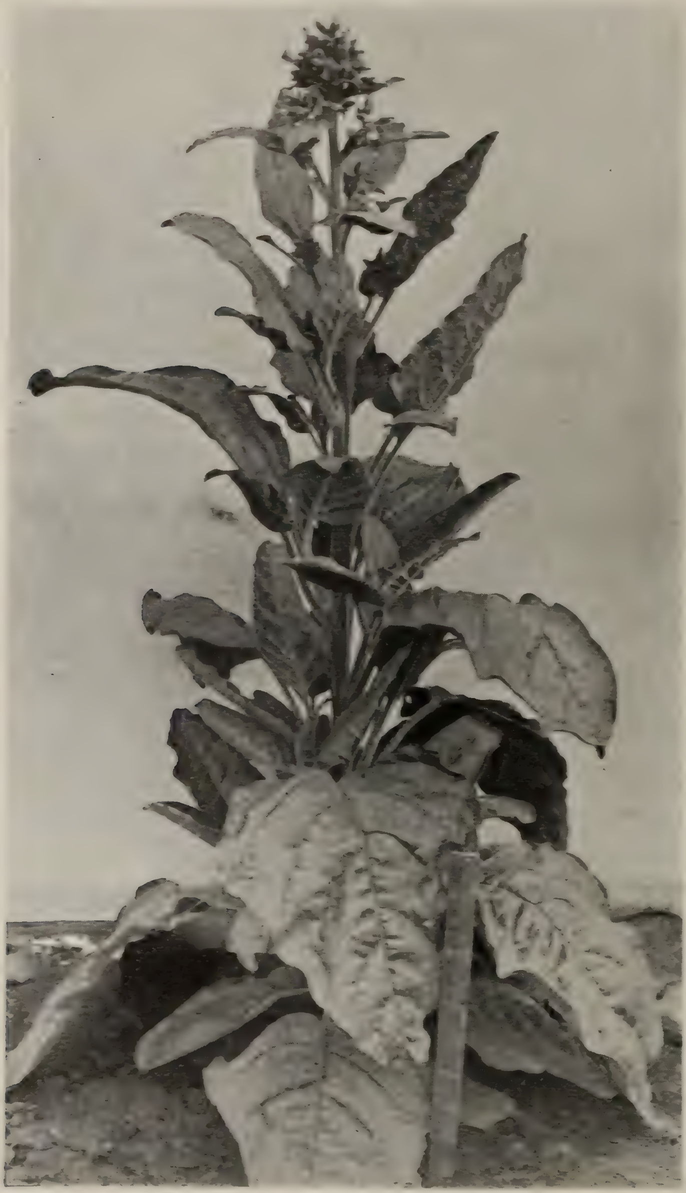

FIGURE 16.—F_2 segregate *N. rustica humilis* × *N. paniculata*. Peculiar habit of growth.

Some few *paniculata*-like characters can be located in a large percentage of the F_2 population. But when one tries to find combinations of these characters, he comes to an *impasse*. There is a gap between *rustica*-like plants with a few traceable *paniculata* characters, until plants appear which are indistinguishable from *N. paniculata*.

Segregation of determiners for fertility occurs in F_1, resulting in certain F_2 plants giving full capsules of viable seed. With seed production taken as the criterion of fertility, the F_2 generation ranges from that of the F_1 generation to that of the pure strains. It may be that individuals inheriting less fertility than those of the F_1 generation appear, but there is no critical evidence that this is true. What may be taken as the facts of the case from the present evidence, is that a frequency curve of fertility on the female side runs from the 3 or 4 percent characteristic of the F_1 generation, to 100 percent, with a marked abmodality toward the side of high fertility. Judgment of the fertility of F_2 by examination of the pollen does not change this general conclusion materially. Adequate evidence of the production of perfect pollen equal to that of either parent,—98 percent maximum, shall we say,—has not been forthcoming, but plants having 96 percent of "good" pollen have been found.

Varietal distinction might be accorded almost everyone of the plants of the F_2 generation in the crosses where *humilis* was used as the *N. rustica* parent. The parental forms were recovered once in every 100 to 200 plants. It should be noted also that of the four plants resembling *N. paniculata* obtained, two were very fertile (one reproducing a characteristic *N. paniculata* population), and two were so sterile that no viable seed was obtained.

Reproductions of all of the named varieties of *N. rustica* were obtained in the F_2 of the cross with *N. paniculata*, though these forms could not all be obtained by intercrossing any two of the varieties themselves.

In the cross between *N. rustica scabra* and *N. paniculata*, about 15 percent of the F_2 generation consisted of delicate dwarf plants of similar type with lack of fertility seemingly correlated with delicate constitution. In the other crosses there were no noticeable correlations between fertility and other plant characters.

Observations on generations later than the F_2 were made only in the case of the cross between *N. rustica humilis* and *N. paniculata*. Three points stand out rather impressively: (1) As judged by F_3 progeny, the F_2 plants show a *much greater degree of homozygosity* than is the general Mendelian expectation in varietal crosses. (2) A selfed plant of the F_2 or of later generations produces progeny with equal or greater fertility than itself. The few cases where a slightly less fertile daughter was obtained, probably may be attributed to physiological reasons unconnected with heredity. (3) Plants almost wholly fertile produce progeny heterozygous for many allelomorphs though the variability of the resulting population, in my experience, is always less (presumably it may be the same) than that of the population from which the parent plant was selected.

In my opinion these are the salient points which should be taken into consideration in seeking a theoretical interpretation of the experiments.

Though certain data were gathered in the years 1916, 1917 and 1919 which make the problem a little clearer, most of the facts reported here had been collected by 1915. In that year I gave a paper before the AMERICAN PHILOSOPHICAL SOCIETY which was reported in abstract in the Proceedings (**54**: 69–72). A provisional hypothesis (A) was rejected.

> "(A) Through multipolar spindles, mating of non-homologous chromosome pairs at synapsis, or other mitotic aberrations at the reduction division, the 24 chromosomes characterizing each of the two species may be distributed irregularly at gametogenesis. If some of these irregular gametes may function, the majority of the experimental data are satisfied, but there are reasons which there is not time to consider which make this scheme improbable."

Obviously any peculiar case of heredity *may* be due to a rare or even a unique distribution of the chromosomes. There may be a doubling of chromosomes as in *Oenothera gigas* and the Primula investigated by GREGORY. An extra chromosome may be found as in *Oenothera lata*. There may be the phenomenon of non-disjunction discovered by BRIDGES in Drosophila. These special cases of inheritance are important, however, only in so far as they show what peculiar results may be brought into line with the chromosome theory of heredity when it is found that the chromosomes do not behave in the usual manner. Such results make ridiculous the attitude of a certain type of scientist, who, when he obtains data he does not understand, immediately proclaims from the housetop that the whole structure of modern genetics has fallen. A special-mechanism hypothesis in interpreting the data presented here has been rejected for four reasons. (1) A special mechanism should never be invoked until a sustained effort has been made to interpret the facts by the method of chromosome distribution we have come to look upon as the *characteristic* type for both the higher animals and the higher plants. (2) Cytological observations in the hybrid plants under consideration warrant us in believing that the machine breaks down in certain cases, but that when it runs it runs in the usual manner. (3) Repeated crosses involving these species having given similar results, and the behavior of other partially sterile species crosses that I have had the opportunity to observe (for example, the wheats) having been of the same general type, it seems likely that a rather general phenomenon is involved. (4) The facts can be given a "regular" Mendelian interpretation with a little subsidiary explanation.

The interpretation favored by the writer and submitted for consideration in 1915 was as follows:

> "(B) On the other hand the facts may be interpreted without assuming irregularities of chromosome distribution if (1) there is a group of chromosomes in each parent that cannot be replaced by chromosomes from the other parent; if (2) there is a group of chromosomes from each parent, a percentage of which may be replaced by chromosomes from the other parent, but where functional perfection of the gametes varies as their constitution approaches that of the parental forms; if (3) there are other chromosomes that have no effect on fertility and therefore can promote recombinations of characters in the progeny of fertile F_2 plants; if (4) a naked nucleus entering the normal cytoplasm of the egg in the immediate cross can cause changes in the cytoplasm that will affect future reduction divisions; if (5) this abnormally formed cytoplasm is not equitably distributed in the dichotomies of gametogenesis in the F_1 generation; if (6) it follows from (4) and (5) that F_2 zygotes may be formed which are less perfect in their gamete-forming mechanism than those of the F_1 generation; and if (7) the heterotypic division of gametogenesis does not necessarily form two cells alike in their viability."

This conception of the matter satisfied the requirements of the data. Each part was added only after careful consideration. But perhaps the statements were too concise to be understood easily. At least no comments, either favorable or adverse, were made on them by other geneticists; and a year later (1916) GOODSPEED and CLAUSEN published an interpretation of the results of some crosses between *Nicotiana sylvestris* and *N. tabacum*, in which they used a conception practically the same as my (1) and (2). This conception they called a Reaction System. The data on which they based their ideas, were briefly as follows. The argument is taken from BABCOCK and CLAUSEN (1918).

Nicotiana sylvestris, a species belonging to the Petuniodes section of the genus, and showing no marked variations after years of cultivation, crosses freely with *N. tabacum* varieties. In any case the F_1 hybrids are uniform in their characters. They are very strong vigorous plants in which the influence of *N. sylvestris* may be seen, but this influence is almost hidden by the dominance [?] of *N. tabacum* characters. Furthermore the hybrids resemble the particular variety of *N. tabacum* used in the cross.

The hybrids are almost sterile, and it is doubtful whether any seeds can be obtained by self-fertilization or sib-crosses. When *N. sylvestris* pollen is used on the hybrids, they set about 1 percent of the normal complement of seeds. The sesqui-hybrids thus obtained show diversity of type; but about 10 percent of them resemble *N. sylvestris* very closely, are almost completely fertile, and give rise to individuals which are seemingly pure *N. sylvestris*. Similarly a cross with *N. tabacum* pollen gives a low percentage of variable sesqui-hybrids approaching *N. tabacum* in character, and those approaching *N. tabacum* most closely are the most fertile.

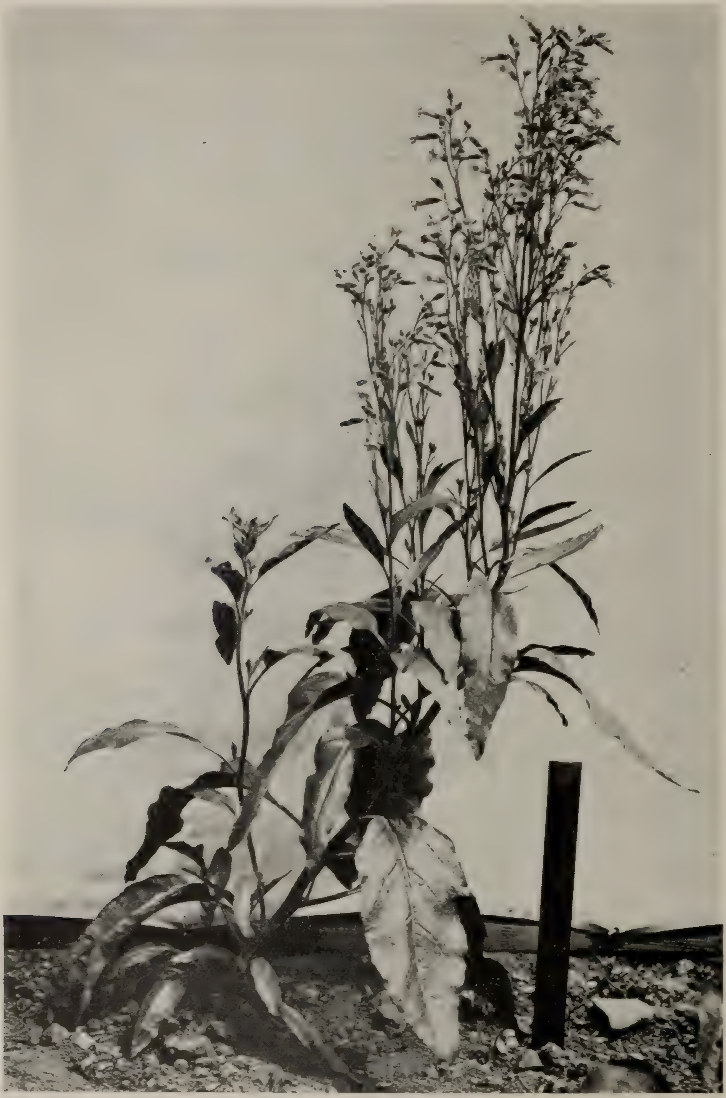

FIGURE 17.—F_2 segregate *N. rustica humilis* × *N. paniculata*, showing characters not found in either grandparent.

In passing it may be said that the writer made this cross in 1908, and the results obtained were very similar to those of GOODSPEED and CLAUSEN.

According to the Reaction-System conception, the normal functioning of a set of hereditary factors depends upon the harmonious interrelations which the factors maintain with each other. In the F_1 hybrids, the resemblance to *N. tabacum* is thought to indicate a more or less complete dominance of the *N. tabacum* factors as a complete Reaction System.

> "Developing the reaction system hypothesis, it would appear that, if the *N. tabacum* and *N. sylvestris* systems display a high degree of mutual incompatibility, any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up between the *N. tabacum* and *N. sylvestris* elements. If the admixture be relatively slight, the inharmonious elements may not greatly affect the workings of the reaction system and there would result individuals showing practically the entire set of characters of one or the other parent, and such individuals would be fully fertile. A slightly greater proportion of inharmonious elements in the reaction system would result in such profound disturbances in its functioning as to produce the abnormal individuals of various kinds which make up so large a proportion of the progeny from such parentage. When the proportions of inharmonious elements in the gametes become still greater, they fail to function at all. It is upon the formation of such non-functional gametes or the attempt to produce them, that the partial sterility of the hybrid depends, and since in this particular case these form by far the greater proportion of gametes, the hybrid is very nearly completely sterile."

BABCOCK and CLAUSEN have given a diagrammatic illustration of the scheme involved here by a table showing the recombination series in the gametes of F_1. It is based on the fact that the haploid number of chromosomes in *N. tabacum* is 24, and on the assumption that the number in *N. sylvestris* is the same. It is reproduced here (table 7) because the number of chromosomes both in *N. rustica* and *N. paniculata* also appears to be 24, and the table will serve as a diagram in our further discussion. The gametic recombinations are given by the expansion of the expression $(1 + 1)^{24}$, the sum of the coefficients being 16,777,316. No crossing over is assumed. The diagram is not meant to be more than a possible illustration of the actual state of affairs in the germ-cells of the hybrid under discussion.

The real issue which GOODSPEED and CLAUSEN wished to call to the attention of geneticists is one to which the latter could scarcely have objection. It is one which has been discussed casually as an *obvious* genetic conception, and yet it has been the basis of criticisms directed against genetic workers by biologists who have been foolish enough to attribute to geneticists the naïve idea of a particulate inheritance in which unit characters, having no better working relation with each other than bricks in a hod, are shuffled and combined at random. GOODSPEED and CLAUSEN deserve thanks for making it clear that geneticists have no such conception, that their real idea is that of a kind of reaction system in which the parts are parts of a smoothly working whole. Organisms may be likened to machines fitted for similar work. The same fuel, the same lubricants, the same paint may be used indiscriminately on either of two samples chosen at random without making much difference in the working efficiency. But let one try to interchange carburetor parts, engine parts, or driving mechanisms, and it becomes a question of whether these parts are closely enough related to be exchanged without upsetting the mechanism in its entirety. Just one misfit of an essential part, and the machine no longer goes.

TABLE 7

Recombination series of gametes of F_1 of Nicotiana species crosses. (After Babcock and Clausen).

CONDITION OF GAMETES	*Tabacum: sylvestris* CHROMOSOMES	PROPORTIONATE NUMBER OF GAMETES	PROGENY WHEN POLLINATED WITH *N. tabacum*	PROGENY WHEN POLLINATED WITH *N. sylvestris*
Functional	24:0	1	Plants resembling the *N. tabacum* parent and of various degrees of fertility	Plants resembling the F_1 and abnormal plants, but all nearly completely sterile
	23:1	24		
	22:2	276		
	21:3	2,024		
	20:4	10,626		
	19:5	42,504		
Non-functional	18:6	134,596	No viable seeds	No viable seeds
	17:7	346,504		
	16:8	736,321		
	15:9	1,307,504		
	14:10	1,961,256		
	13:11	2,496,144		
	12:12	2,705,456		
	11:13	2,496,144		
	10:14	1,961,256		
	9:15	1,307,504		
	8:16	736,321		
	7:17	346,504		
	6:18	134,596		
Functional	5:19	42,504	Plants resembling the F_1 hybrid and nearly completely sterile	Abnormal, infertile plants and fertile plants closely resembling *N. sylvestris*
	4:20	10,626		
	3:21	2,024		
	2:22	276		
	1:23	24		
	0:24	1		

With this idea we are wholly in agreement, but we wish to go a little further and try to picture the reproductive mechanisms of the plants involved, in such a manner as to satisfy the known facts of our investigations.

In the first place let us consider the F_1 generation of the cross between *N. rustica humilis* and *N. paniculata.* The two parental strains having shown a very high degree of uniformity, one must assume that each is homozygous for practically all hereditary factors, that every gamete of each type has the same constitution, and that all of the F_1 zygotes are therefore alike. These assumptions fit in with the fact that the F_1 population showed no more variability than the parental populations. Again, one must assume that the cytoplasm of the germ-cells of the parents has little effect on the actual characters of the F_1 generation, because nearly all, and possibly all of the cytoplasm is furnished by the maternal parent, and the reciprocal F_1 generations are to all intents and purposes identical. But there is no reason why one cannot suppose that the cytoplasm helps lubricate the machinery of the reduction division, so to speak.

Turning now to the gamete production of the F_1 generation, let us glance at table 7. Manifestly the actual production of gametes falls far short of the theoretical production possible when none is aborted. But do the pedigree-culture facts and the cytological observations give us any reason for concluding how and why this abortion occurs? Let us see. Clearly the obvious suggestion is the one we have given in B (2) to the effect that there are "chromosomes in each parent that cannot be replaced by chromosomes from the other parent." As GOODSPEED and CLAUSEN would express it, certain chromosome combinations will fail to function because the reaction system has been upset. The simplest diagram of the situation is that suggested in table 7 where the 6 chromosome combinations at each extreme carrying 19 to 24 chromosomes of one parent and 1 to 6 chromosomes of the other parent are the only ones functioning, thus providing for about 7 functional gametes per thousand. The difficulty with this diagram is that it is too simple. The proportion of functional gametes is at least 30 per thousand, judging from the fertile ovules. In individual capsules it ran up to about 100 per thousand, but this high figure may have been a distorted result caused by insufficient samples. With due regard for the theory of random sampling, however, 30 per thousand is somewhere near the maximum limit. Of course the proportion of functional gametes appeared to be much less, but part of the sterility exhibited there may have been due to causes that had little to do with selection according to hereditary constitution. It seems to me that this is a fair deduction from the fact that reciprocal back-crosses produced about the same amount of seed. To fit these facts, therefore, one must suppose that from 20 to 24 chromosomes of one of the parents must be present in the functional gametes produced by the F_1 generation.

Now the above assumptions will account for the sterility found in the F_1 generation, for the variability exhibited in the F_2 generation, and the frequent appearance of types like the grandparents which appear there. If a differential compatibility is assumed at the ends of the table, i.e., if more of the *paniculata* chromosomes must be present on the one hand than of the *rustica* chromosomes on the other hand, in order to produce functional gametes, the *rustica*-like appearance of all but a few F_2 individuals is accounted for. In fact one should assume functionality in the first case only when either 23 or 24 chromosomes of *paniculata* are present. But with these simple assumptions one does not fully satisfy the following conditions found in the F_2 generation, viz., the appearance of plants identical with other *rustica* varieties, and the appearance of homozygous individuals with great frequency.

These two phenomena seem to be related phenomena of considerable significance. Do we not have here an addition to the steadily accumulating evidence on simple Mendelian unit characters, that changes in the germ-plasm are largely changes in organization which affect only an end result? Here, for example, one finds in *N. rustica humilis* a variety capable of producing plants having certain definite characters. But when crossed with *N. paniculata*, the other well known varieties of *N. rustica* appear in the F_2 generation. Is this not an indication that in the germplasm of *N. rustica humilis* there exist the potentialities of producing all of the other varieties waiting only the chance for a particular reorganization which comes when the cross with *N. paniculata* is made? To the writer's mind the simplest interpretation of the appearance of these types and their appearance in relatively homozygous conditions, is that *N. rustica* and *N. paniculata* in their evolutionary divergence from each other have not varied in all of their chromosomes. One might suppose, for example, that among the 24 chromosomes of the two species, 12 are identical in composition, and therefore in function; 6 others differ in composition (unit factors) very slightly; while considerable evolutionary divergence has come about in the remainder. It is not necessary to draw a concrete picture of the results to be expected from these assumptions. It is clear that any degree of gametic sterility necessary to satisfy the experimental evidence can be obtained, that one can obtain many different types in F_2 and that many of these types will be to a high degree homozygous.

With these facts in mind, the postulates of B (1), B (2) and B (3) will stand, provided that in B (3) some of the chromosomes having no effect on fertility are assumed to be identical in the two species. The postulates B (4) to B (7) were made to interpret what at that time was thought to be a fact,—recombination which gave plants of the F_2 generation *less* fertile than those of the F_1 generation. There is a grave difficulty in the way of interpreting such behavior on the assumption that difference in genetic constitution is the basic cause of sterility. The greatest degree of genetic difference, heterogeneity if you will, exists in the F_1 generation. Recombinations, whether by the shuffling of whole chromosomes, or by crossing over, increases homozygosity. *A less degree of genetic difference in the F_2 generation*, therefore, cannot be *the cause of greater sterility* than was exhibited by the F_1 generation. For these reasons, we interpreted the matter by assuming some sort of a mechanical control of the process of reduction by the cytoplasm, a cytoplasmic lubrication of the process, so to speak. Building on this foundation, increased sterility in the F_2 generation can be explained very clearly by the four postulates B(4) to B(7). Under our original presumption of fact, all of them are necessary, and none is on its face improbable. But since the evidence of lessened fertility in any of the individuals making up the F_2 generation is inadequate, and the proportion of individuals where a possible lessened fertility exists is very small, these hypotheses may be discarded as unnecessary. Lessened fertility in F_2, if it exists at all, may be the result of special conditions unconnected with our assumption of the general correlation of sterility with a high degree of genetic heterogeneity. It can be interpreted by the assumption that special factor combinations enhance sterility. For example chromosome pair $\frac{AB}{AB}$ in one parent and chromosome pair $\frac{ab}{ab}$ in the other parent may function in such a way as to provide for fertility. The F_1 combination $\frac{AB}{ab}$ may also be fertile. But the combinations $\frac{Ab}{Ab}$ and $\frac{aB}{aB}$, possible after the formation of crossover gametes, may be sterile.

If we are to make a general summary of the results of this work, then, it will be an extension of certain conclusions reached by EAST and HAYES in 1912. In this paper a series of crosses between various Nicotiana species were discussed from the standpoint of their behavior as first-generation hybrids. Vigor of heterosis was seen to accompany hybridization far beyond that point of difference between the species entering into the combination which produced sterility. Then came a degree of difference which accompanied a sudden drop in vigor to a point below that of either parental type. The conclusion was that at a certain point differentiation in gametic structure is so great that the reduction division with the formation of all the possible types of gametes does not take place in the normal manner. At this point of differentiation, however, ontogeny may go on normally. With a still greater differentiation in gametic structure even ontogenetic cell division does not proceed in the usual manner, and a marked lowering of vigor occurs. Accompanying a constant increase in the constitutional differences exhibited by the species entering into the hybridization, as assumed to exist from the behavior of the crosses, comes a constantly greater deficiency in ability to go through the life cycle, the last stage before total incompatibility is reached being the production of seeds having a partly developed endosperm but no embryo.

These experiments carry one of the ideas expressed at that time a little further. Numerous matings were made between two species in which differentiation had gone just far enough to cause the great majority of the gametes to cease to function; yet in all the behavior of the pedigree cultures and in all of the cytological observations, the evidence is that inheritance proceeds in a normal Mendelian manner. With similar gametes uniting to produce an F_1 generation, great uniformity results. Ontogenetic division has proceeded regularly in spite of the unlike constitutions of the two haploid sets of chromosomes possessed by each nucleus. When gametogenesis occurs in the immature germ-cells of the hybrids, the evidence is that it also takes place in a wholly normal manner, but that only a part of the resulting gametes are functional. What is more natural than to suppose that the gametes eliminated are those in which chance has thrown together such a nearly balanced lot of chromatin of different constitution that proper reorganization in preparation for their essential gametic functions is impossible? And the evidence of the pedigree cultures bears out this view.

Other inductions may be made from the behavior of the cultures, however, without drawing upon the imagination. In the first place, it is clear that if the assumption as to the cause of sterility be granted, more chromosomes of *N. paniculata* are necessary for proper functioning of the germ-cells when they are in the majority than when those of *N. rustica* provide the excess. Secondly, the variability of the F_2 generation, the peculiar types produced by a small number of F_2 seeds, and the behavior of the F_3 generations produced by self-fertilized F_2 individuals, make it seem probable that certain heterozygous combinations are formed but are eliminated as zygotes. It may be asked why, if the F_1 individuals go through their development normally when they must of necessity possess the greatest degree of heterozygosity, F_2 individuals should be eliminated? The question is pertinent, but not unanswerable. The gametes of the parental strains are formed under natural conditions. They are perfect gametes. When fertilization occurs ontogeny proceeds in an orderly manner, and abnormal conditions are manifested only at gametogenesis. At gametogenesis some of the gametes expected are formed, others are eliminated. Does it not seem probable then, that a certain proportion of those functional gametes formed by the F_1 plants are still so abnormal in structure that when fertilization occurs development is difficult? Now it seems wholly reasonable to assume that difficulty of development is in this instance correlated with heterosis, i.e., with unlike chromosomes, just as difficulty in gametogenesis is similarly correlated in the F_1 generation.

It is further probable from the ratios obtained, that a considerable percentage of the chromosomes of the two species have not been differentiated in constitution, or have been differentiated very slightly. In fact this last assumption appears to be the simplest way of accounting for the occurrence of the duplicates of varieties *brasilia*, *scabra* and *texana*, in the cross with *N. paniculata* where only *N. rustica humilis* was used.

Finally, there is a rather peculiar circumstance for which to account in the comparatively low variability of the back-crosses of the F_1 generation with either parent. In varietal crosses similar matings give a less degree of variability than a true F_2 generation, but the extreme variability of type of the F_1 gametes is unquestionably brought out. Here the variability, though considerably greater than that of the true F_1 generation, is not such as would appear to be possible if all of the F_1 gametes functioning in the production of a true F_2 generation were to unite with the gametes of the pure species and produce viable zygotes. Confessedly, this phenomenon is somewhat of a puzzle. I can see in it only a further elimination of the extreme gametes of the F_1, but just why such a peculiar thing should occur, is not clear. Perhaps our observations are distorted in that we had become accustomed to an inordinate variability in the combinations of gametes forming the F_2 generations, and had expected a greater variability than was proper when a series of like gametes mated with those of the F_1 generation. Possibly a variability standing half-way between that of the F_1 generation and the F_2 generation was not to have been expected. Certainly the variability exhibited by the back-crosses was more that of an F_1 generation than one would suspect would be the midpoint between the F_1 generation and the F_2 generation. But this may simply be an erroneous idea.

Such, then, are the theoretical conclusions to be drawn from the data. But there is also a conclusion of practical significance which ought not to be neglected. Not only these experiments but other critical experiments on plants indicate that the greatest opportunity for plant improvement and possibly for animal improvement as well, lies in crossing together types which have become as far differentiated from each other as is possible without producing complete sterility in the hybrids. When species differing in a large number of allelomorphs can be crossed, and the resulting hybrids are completely fertile, there is a wonderful chance for desirable recombinations occurring in the F_2 generation. In cases of this kind that we have studied, however, the appearance of the F_2 individuals and their behavior on further breeding indicate a large number of *minor* variations in the chromosomes. The variability of F_2 is great, it is true, but recombination of a great number of factors having a minor effect on the morphology or the physiology of the individual, gives a graded series difficult to distinguish the one from the other. Opportunity for selection is there, but with so many individuals massed in the intermediate classes, practical use of selection is rather difficult. On the other hand, when two species are still further apart in their constitution, so far indeed that partial sterility becomes characteristic of the F_1 individual, the chromosome differences produce marked results even though the great majority of the gametes and zygotes is eliminated. That is to say, the greater the differentiation of the protoplasm the greater the chance for extreme differentiation in the individuals which do pass through the selective sieve that eliminates so many. Therefore one may assume that the greatest opportunity for obtaining new and desirable domestic forms is by crossing types as different from each other as is possible with some degree of fertility in the F_1 generation.

Again, we believe that just those families and genera that have varied widely in nature, yet have retained that necessary physiological compatibility which allows them to cross and to produce more or less fertile hybrids, are the ones—the only ones—that have been *really important* as domestic plants and animals. Some of the genetic evidence, and most of the historical evidence relating to the important cultivated species indicates a polyphyletic orgin. Such critical data as we have, therefore, indicate that it was possible to domesticate, to improve and to use the forms man has utilized, just because nature had prevented too great sexual incompatibility from accompanying the physical differentiation that had arisen in certain genera through natural selection. In fact we believe this conclusion has the dignity of a natural law; viz., the desirability of a type for domestication varies directly with the amount of physical variation and inversely with the amount of sexual incompatibility accompanying such variation. We believe that plant breeders and animal breeders should emphasize this more and more in a practical way, that they should never lose sight of the fact that conscious selection of valuable types of organisms is a useful tool only when there is material for selection. And crossing, wide crossing, furnishes the greatest opportunity to obtain this material.

It has been the writer's experience that the evidence as to the polyphyletic origin of the great groups of domesticated plants and animals, based on historical and anthropological grounds, is not fully appreciated. When this evidence is compared with the genetic evidence of compatibility at hand, there is really an extremely good case *a priori* for asking the government to undertake practical plant breeding and animal breeding through hybridization on a much greater scale than is going on at present.

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GENETICS, JULY, 1921

TABLE OF CONTENTS

Page

E. M. East, A study of partial sterility in certain hybrids.......... 311

J. C. Phillips, A further report on species crosses in birds.......... 366

Harry H. Laughlin, Dice-casting and pedigree selection. Experiments which picture mathematically close analogies between dice-casting and certain breeding phenomena................. 384

Karl Sax, Sterility in wheat hybrids. I. Sterility relationships and endosperm development................................. 399

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A number of subscribers have made inquiry concerning the indexes for volumes 3, 4 and 5 of Genetics, and it is fair to assume that other subscribers are interested in the same question. The Editor is alone responsible for the delay in the preparation of these indexes, and inquiries should be addressed to him, therefore, rather than to the publisher. The three indexes are in various stages of progress and will be brought to completion as rapidly as circumstances will permit. When each is completed it will be bound in with the then current issue of Genetics, so that subscribers can not fail to receive them as soon as they are published. It is hoped that this notice will make further correspondence concerning these indexes unnecessary, as every additional letter that must be answered requires time that might be devoted to the preparation of the indexes.

THE AGRICULTURAL LIMITS OF OUR POPULATION

By Professor E. M. EAST
HARVARD UNIVERSITY

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[Reprinted from The Scientific Monthly, June, 1921]

THE AGRICULTURAL LIMITS OF OUR POPULATION

By Professor E. M. EAST
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RECENTLY Pearl and Reed[1] have shown that growth of population in any country is expressed by a portion of the curve of a logarithmic parabola having the formula $y = \frac{be^{ax}}{1\text{-}ce^{ax}}$, where a, b and c have postitive values. In developing this law of population increase, the authors have been guided by true biological premises, and have made the first original contribution to the problem of population since the time of Malthus. The fit obtained when the records of the United States Census are applied to this curve is truly remarkable, as indeed are the fits of census figures of other countries; and any student of population hereafter who does not give careful attention to the conclusions they have drawn, is treating his subject in a superficial manner.

The particular point in this paper to be discussed here is the major asymptote of the curve as applied to our own country. This gives the ultimate population limit of continental United States as 197,000,000, a figure so much smaller than has usually been predicted that it has aroused much comment. Doubtless Pearl and Reed realize how hazardous it is to predict. One who makes a prediction after a careful study of a situation is immediately bombarded with impromptu criticisms submitted by those who have made no such study, but who feel that in their infinite wisdom they have immediately thought of numerous variables left out of consideration by those against whom their criticism is directed. Thus it will be said that errors in the census figures will change the end result, that methods of agriculture will improve radically, that standards of living will be simplified, that new methods of utilizing energy will be found, that synthetic foods will be produced, that any one of a hundred things will occur which will make it possible for a much greater number of persons to be enlisted under our star-spangled banner than Pearl and Reed find to be probable under existing tendencies. Such criticisms are especially likely to appear on the subject of population, for a great many persons seem to take it as a direct personal insult when one suggests either the advisability of a population limit as a factor in national welfare or the possibility of a population limit as the result of natural laws. To limit anything in the United States is blasphemy, though what the particular pleasure is in contemplating the country as an immense box of sardines, I am unable to discover. The authors would no doubt admit the possibility of certain changes in the present tendency for the population to increase. They would grant that for this reason the upper limits of our population are not to be set in 1920 with any high degree of accuracy. At the same time it could be shown, if space permitted, that criticisms of the type suggested above are not very cogent. They are the suggestions of the dilettante. The biologist realizes the improbability of new food products because of the fact that all our important foods were brought into use by prehistoric man. The geneticist is very conservative in his estimation of the prospects of a really great improvement in the domestic plants and animals. The chemist admits the possibility of synthetic foods as a laboratory stunt, but points out that as a practical scheme it is almost analogous to perpetual motion, since energy must be used up and man has never been able to approach nature in utilizing energy. The agriculturist points out the meagre changes in the art of agriculture which are the product of our civilization, and notes that those changes which have occurred are merely vehicles for exploiting a limited store of soil fertility at greater and greater speed. If there is any realm of activity wherein there is a small probability of radical future changes, it is agriculture; if there is any field wherein prediction for the future can be made from a study of the past with less likelihood of error, it is that in which agriculture is the major variant. Therefore a figure of 197,000,000 as the ultimate limit of population for the United States can not be cast aside for a *priori* reasons.

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[1] Pearl, R. and Reed., L. J. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci. 6: 275-288. 1920.

What the writer proposes to do in this paper is to show that the agricultural possibilities in this country are not so great as they are generally supposed to be, and that even if these possibilities were to be fulfilled wholly the population the country could support is not enormously greater than the figures Pearl and Reed have set. And naturally one has no good reason for saying the upper limits of food production will be reached because of population pressure. It is to be presumed that Americans will have sufficient foresight to adjust the birth rate to the death rate and to attain a stationary population without the compelling force of grim necessity. We shall not discuss the possibilities latent in new food products, in better cultivated plants and domestic animals, or in new methods of agronomy. They have been considered carefully, and it is our opinion that they are not important factors in the matter.

In an essay on population published in a previous issue of this journal,[2] the writer showed what a comparatively small amount of reserve agricultural land still exists in the United States from which to produce food for our increasing population—a population which is rising by leaps and bounds. Unfortunately our reference to rapid population increase has been misunderstood in various quarters. This misunderstanding was caused by our calling attention to Pritchett's calculation of our future population based on the supposed fit of our census returns to part of the curve of a third order parabola. It was obvious from its shape that this curve had no biological basis, and the absurdity of expecting 386 million people in the year 2,000 and 1,113 million in the year 2,100 was pointed out. The figures were used merely to bring out the terrific rate of increase maintained at the present time, and to emphasize the efforts necessary to provide food if this increase were to be maintained. The amount of reserve agricultural land not now in farms proved to be about 35 per cent. of the present farm area. Since in the nature of the case this uncultivated land must be of poorer quality on the average than that which has been cultivated in previous years, we are warranted in assuming that even less than 35 per cent. of reserve land remains in this country when considered on a productivity basis. These calculations were made carefully and were sufficiently accurate for the purpose for which they were used, viz., to show how rapidly our population was encroaching on our potential food supply.

Since this paper was written a detailed study of the "Arable Land in the United States" has appeared under the authorship of O. E. Baker and H. M. Strong, of the United States Department of Agriculture, as one of the papers in the 1918 Yearbook issued in 1919. These authors have made an extremely valuable investigation of the agricultural lands of the United States, and support their conclusions with adequate data. We wish to point out some conclusions as to the possible ultimate population of the country with their figures as the basis. We venture to make only two slight changes. The first change is to withhold permanently from farm use an additional 50 million acres of potential improved land to provide space for future cities, roads and railroads. The second change is a reclassification of the land, which, because of its arid nature or because of its topography, can never be used for primary food production. Baker and Reed have classed only 38 million acres out of 653 million arid acres as desert having no grazing value. But as a matter of fact about 200 million acres more are desert lands where the grazing value is negligible.

The final disposition of the lands of the United States, then, may be expected to be somewhat like this: improved land, 800 million acres; forest and woodland 360 million acres; range land and unimproved pasture 415 million acres; desert 238 million acres; cities, roads and railroads 90 million acres. One thus accounts for the 1903 million acres making up the total land area of the country.

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[2] East, E. M. Population. Sci. Mon. 10: 603-624. 1919.

Those who cast a distrustful eye on the 1103 million acres, 58 per cent. of the total land area of the country, which are set apart as land which must be permanently withheld from cultivation, are referred to the arguments given *in extenso* by Baker and Strong. The reasons are plainly set forth in their paper, and if the writers err they err on the side of optimistic conservatism. Their figures may have to be revised at the end of a few centuries, but it is more likely that they will be revised upward than downward.

In brief, 743 million acres of these lands are set apart because of natural irremediable conditions. They consist of arid lands having no possibility of irrigation, lands where the mean summer temperature is too low to grow crops, lands where the topography is such that cultivation is impossible, lands which are without natural soil fertility, and undrainable swamps. Nevertheless such lands, when the population pressure becomes sufficiently great, may be used to produce some food. The cheerful optimist, and we shall take on that rôle for the time being, may perhaps assume that there is the possibility of supporting one person per each 100 acres upon them—a total of 4 million people.

The 360 million acres of forest and woodland must be retained for a variety of reasons. In most cases their topography prevents their cultivation, so that they could not be used for farm lands even if no other obstacles intervened. But beyond this fact stands the opinion of meteorologists, that if this figure for a forest reserve be cut, a change in the rainfall and the temperature of the improved land would ensue such as to reduce its productivity much beyond that of any increased production resulting from the additional new lands. On the other hand, there remains the possibility of producing considerable amounts of both primary and secondary foods on these reserves without destroying their usefulness as forests and woodlands. When food becomes sufficiently scarce and the price in consequence becomes sufficiently high, these lands will be utilized. They will perhaps produce sufficient food to support one person for each 50 acres, giving an additional potential population of a little over 7 millions.

There remains, then, a potential 800 million acres of improved land for consideration. This figure is made up by adding together the 478 million acres in cultivation in 1910, 30 million acres of desert land which irrigation engineers believe may be irrigated, 60 million acres of swamps that can be drained, 82 million acres suitable for dry farming and upland pasture, and 200 million acres of tillable land which is now in woodland. The sum obtained is 850 million acres. But from this sum must be subtracted a parcel of 50 million acres to allow for the growth of cities, roads and railroads. 800 million acres of arable land are left. The question then arises; what potential population will this land support? It is a question that can be answered in a rough way by comparing the per capita acreage of cultivated land in foreign countries as compiled by the International Institute of Agriculture at Rome.

Before the war Germany cultivated 48 per cent. of her land area, France cultivated 45 per cent, Italy cultivated 48 per cent, and Belgium 49 per cent. It will be seen that these figures are fairly comparable with the 42 per cent. proposed for the United States as the sum total of her arable possibilities. Furthermore our comparison is fair in a historical sense, since these countries were over-populated as shown by the fact that they did not live within themselves agriculturally, and since our own country is made up of peoples from similar racial stocks and therefore may be presumed to have similar tastes, customs and abilities.

On the face of the returns, these countries were tilling the following number of acres per capita; Germany 1.15 acres, France 1.5 acres, Italy .98 acres, Belgium .57 acres. These figures, however, do not show the true state of affairs. For example, the total arable land of Belgium can not be compared with that of the United States. It is much too good. It is no more right to use her wonderful bottom lands as a standard than it would be to use the Nile overflow lands of Egypt where nature robs Peter to pay Paul and produces greater crops than anywhere else in the world. It is no more proper than to expect all the soils of the United States to come up to the production figures of the Mississippi Valley. Exclude Belgium and a fair comparison can probably be made if one is careful to make the comparison fair. These countries were not self supporting. Huge quantities of food were imported annually. We have not been able to obtain the complete returns for exports and imports from which to make an accurate accounting of the number of people actually supported per acre in such countries as France and Germany where both the standard of living and the efficiency of the agriculture is high. In Germany it seems to have been pretty close to 2.2 acres per man when all corrections are made. In France it was probably somewhat higher. Personally I believe that 2.5 acres per man is the limit that we may hope to reach in the United States if we cultivate our whole 800 million acres, if we keep up a reasonable standard of living, if we follow rather closely our present food habits, if we conserve carefully our soil fertility, and above all if we farm as intensively, as scientifically and with as little waste as the best farmers of Europe who have been under the necessity of perfecting their agriculture because of population pressure during the past half-century.

If this assumption be true, our 800 million arable acres will support 320 million people, a figure to be added to the 11 millions obtained by calculating the productivity of our grazing lands and forests. The maximum population the United States can support under any conditions conceivable to those of us who live at the present day, therefore, is 331 millions.

To be sure 331 millions of people is a right tidy little population. China is supposed to have about 300 millions, with a land area 600 million acres greater than the United States. Can we wish to be placed ultimately in an economic position less favorable than that of present day China? We should all answer, No! Is it, then, improbable that we shall use a little forethought before this time comes, and establish a birth rate adjusted to the death rate which will withold from us the economic pressure which she confronts at all times? If this be reasonable, is it not probable that Pearl and Reed's figures of 197 million, as the upper limit of the population that the United States will actually attain, are wholly just and proper?

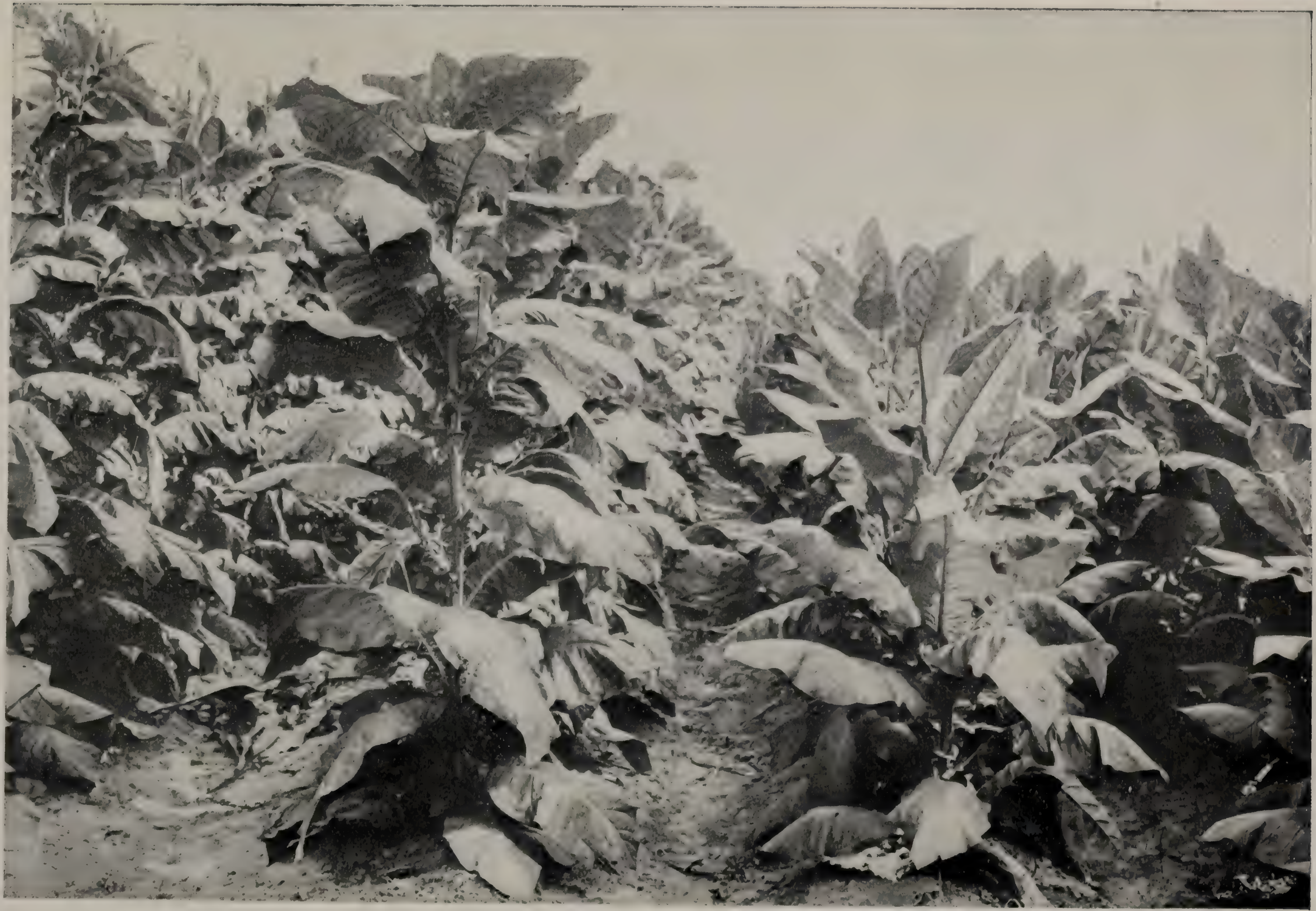
The most optimistic of our population enthusiasts will criticize these figures no doubt. They will point at once to Japan as the shining example of efficient agriculture, an example that we ought to hold ever before us and strive to emulate. Japan, with a population of 52 millions in 1911, cultivated 18 million acres out of a total land area of 94 million acres. By an extraordinary use of fertilizers, which reached a figure of over 4.5 tons per acre over the whole cultivated area, exclusive of commercial chemicals, she was thus able to support 3 persons per acre. In reality the figures reported should not be quite so high, as Japan imported a very much larger amount of foodstuffs than she exported in the years just previous to the war. But with due allowance for imports Japan did actually produce food sufficient to support about 2.5 persons per acre of cultivated land. If the United States could do as well on her 800 million acres of arable land, our population might have a maximum limit of 2000 millions—a figure greater than the present population of the world.

Let us stop a moment and do a little more figuring before we accept this computation. The Japanese are small. Their army rations are only about one-half of ours. Their population as a whole gets along with less than half of the food per capita required by the people of the United States. Undoubtedly our own people are great wastrels because they have more to waste, and could get along with less if it were necessary; but it may be questioned whether Japan's crops would support more than 1.5 Americans per acre, even with a material lowering of the standard of living and a higher standard of efficiency for food utilization. Naturally it will be maintained by some that this difference in food consumption between the American nation and the Japanese nation is unnecessary. It will be pointed out that excess of body weight in one over the other is not over 25 per cent., and that for this reason a 25 per cent. difference in food production is all that can reasonably be demanded. But this is not the whole truth in the matter. Food habits are very ancient. Pearl has shown that the people of the United States did not reduce their food intake during the war though there was an urgent moral necessity that this should be done. Nevertheless during this period the people not only ate just as much, they even demanded their food in whatever expensive forms appealed to their tastes. Our contention is, therefore, that while a constant economic pressure maintained over long periods may modify our food habits, it will be just as difficult for us to cut our present ration as for the Japanese to cut the one to which they have become accustomed. And it does not appear that such established folkways will be materially modified in the comparatively short length of time which present indications allow us as the interval before we reach a close approximation of our population limit—i. e., the point where our population curve turns sharply toward the horizontal.

There is still another important modification of our computation to be made. We have an extensive system of agriculture in this country. Our yields per acre are comparatively low; but we farm more land per man than any other country. The Belgian, the most intensive farmer of Northern Europe, for example, cultivated 5 acres per man before the war; the comparative figure for the United States is 26 acres. Now we have calculated that the United States contains 800 million acres of arable land that can ultimately be put under the plow, but if we suppose that this 42 per cent. of the whole land area of the country can be made to produce the crops which Japan produces on 18.7 per cent. of her area we are making a grave mistake.

Japan cultivates a comparatively small proportion of her land area by a method built up in China, because she has found that she thus gets the greatest returns. She even goes so far in her conservation and utilization of soil fertility as to strip the surface soil from less productive areas for use upon her best land. Indeed it is wholly probable, as may be seen by a study of the relief maps of the two countries, that Japan has almost as high a percentage of arable land as the United States. But she has found that it does not pay to try to cultivate it all. We have considerable reason for supposing that Japan would not obtain a significant increase in her total production of foods if she cultivated 42 per cent. of her area instead of 18.7. If, therefore, her productiveness were measured in relation to the first percentage (42), she really would need 1.5 acres per man to support her population in terms of American food habits. At this rate, the United States could support only 530 millions on her arable land. Presumably this figure may be taken as the ultimate limit humanly possible for the country. That we shall ever reach this figure seems to the writer wholly absurd.

Reprinted, without change of paging, from the Journal of Heredity (Organ of the American Genetic Association), Vol. XII, No. 2; Washington, D. C., February, 1921



ROUND TIP TOBACCO AT THE LEFT COMPARED WITH HAVANA TOBACCO AT THE RIGHT

Here is a good illustration of the plant breeders' art. Three essentially different requirements were met in the production of a new tobacco now known as Round Tip which produces the finest cigar wrappers in the United States. The manufacturers and consumers of cigars have thus been satisfied as well as the growers who demand a strong, many-leaved, disease resistant plant. The new hybrid is shown at the left in the photograph. Compare its leaves with those of the Havana plants at the right. (Frontispiece.)

ROUND TIP TOBACCO—
A PLANT "MADE TO ORDER"

From Specifications Drawn by Manufacturers and Consumers of Cigars, and the Growers of Tobacco, a New Plant is Grown to Satisfy the Demands of Commerce.

E. M. East,
Bussey Institution, Harvard University, and
D. F. Jones,
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A NEW type of cigar wrapper tobacco known to the trade as Round Tip, has given such evidence of commercial success that its origin may be of some interest, particularly as it is one of the few instances where a plant was actually made to order with the specifications drawn beforehand.

The manufacturer of cigars, knowing the demands of the consumer, calls for numerous qualities in the leaf he uses to enclose his wares. Primarily of course, it must burn rather freely—but not too freely—and must leave a gray-white ash which does not flake off at each puff of the ultimate consumer. The flavor must be of that peculiar character acceptable to the connoisseur, which may be described as neither earthy nor bitter but somewhere in between. Possession of the delicate aroma so dear to the heart of the confirmed smokers is not essential. The aroma of a cigar is furnished by the leaf used as filler; but the wrapper must have an agreeable aroma or none, since the most delicately perfumed filler ever produced may be utterly ruined by a nauseous wrapper. It must have a velvety texture, and a uniform color—not too dark nor yet too light. It must be greenish-brown rather than reddish-brown, and above all must not be coarse veined or show white markings around the veins.

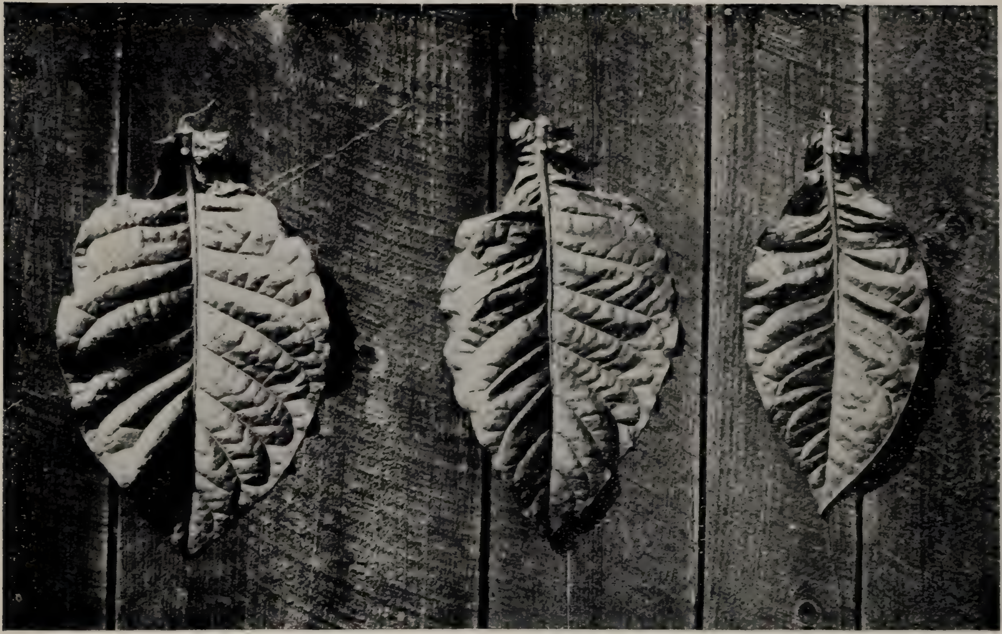
THE MANUFACTURER'S SPECIFICATIONS

So much for the demands of the consumer. Comes now the manufacturer, pressing his needs. Cigar wrappers cost money, and he must have a thin leaf covering as many cigars as possible per pound of material. The leaf should be broadly rounded at the tip, since the wrapper of finest quality comes from this portion of the blade. Again, the cigar wrapper must be strong and full of elasticity. It shaves down the profit to have a significant percentage of torn leaves come from the wrapping bench. If the purchaser breaks the wrapper in his pocket, all very well, provided he doesn't do it often enough to prevent his coming back for more; but broken leaves in the factory break the heart of the manufacturer.

THE GROWER'S DEMANDS

Finally, enter the farmer. His demands are always last to be considered, since the common practice in our national drama is to cast the farmer in the rôle of "goat" instead of giving him the star part he deserves. The grower demands a plant of quick maturity, a plant with many leaves, a plant with no lateral branches. His ideal is a strong plant standing up under wind and rain, a plant resistant to disease, one which will fill his barns to overflowing and cut down the unit growing cost.

In the narrow basin of the Connecticut river, on the land between Holyoke, Massachusetts, and Glastonbury, Connecticut, they grow the finest cigar wrappers in the United States. And this is really quite an odd fact. The two regions most famous for cigar wrappers are Sumatra and Cuba. The first is astride the Equator; the second is just south of the Tropic of Cancer. Then away up at forty-two degrees north latitude is this spot bidding fair to rival them, a place where soil and climate again combine to make matters right for the sovereign weed. Here some 35,000 acres are grown which bring the planters higher returns than



LEAVES OF SUMATRA TOBACCO—ONE OF THE PARENTS OF ROUND TIP

Sumatra and Cuba are the two most famous regions producing tobacco for cigar wrappers. Sumatra tobacco, while grown in the Connecticut valley, does not compete commercially with the other varieties. It, however, possesses some most desired and promising qualities, and was used as one of the parents of Round Tip. The illustration shows bottom, middle and top leaves from a Sumatra plant. (Fig. 1.)

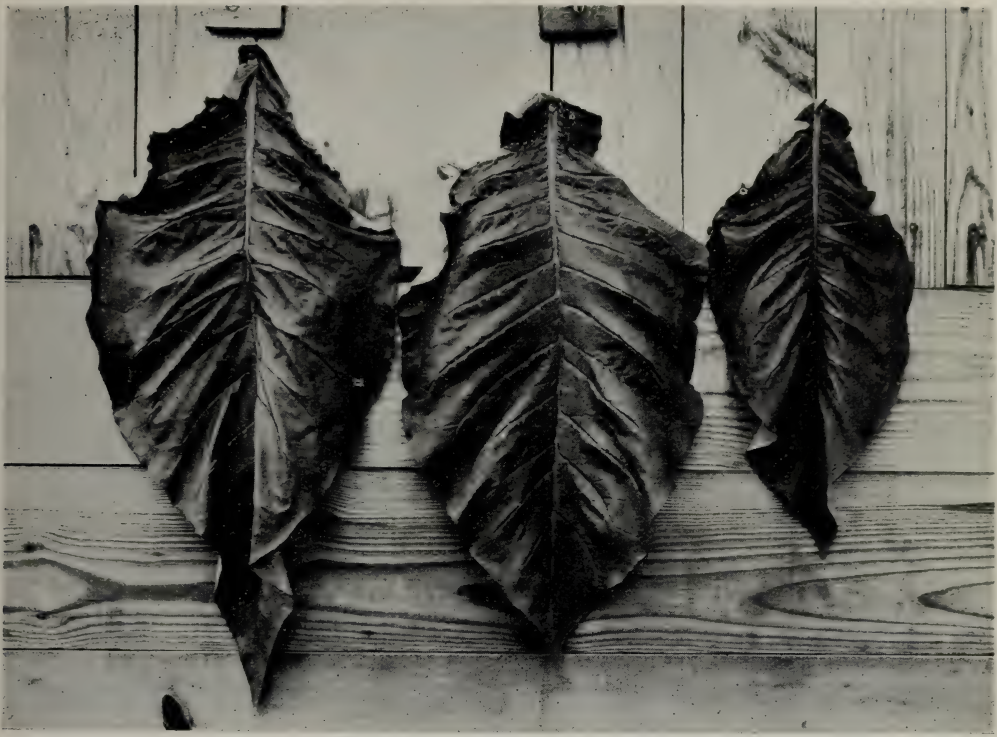
any other place of equal area on the globe.

FOUR DISTINCT VARIETIES OF TOBACCO IN THIS DISTRICT

The Connecticut valley confines its attention to three markedly different types known as Cuban, Havana seed and Broadleaf. A fourth type, Sumatra, has been tried commercially at various times, but has not been able to compete with the other varieties. It must be considered in our discussion, however, for it has certain qualities not possessed by the others.

Four tobaccos then form the basis of the nicotinous wealth of this little district. Unfortunately, like most mundane objects, each has its defects. The Sumatra wrapper leaf, though very popular with the manufacturers in this country, is small, thin and tasteless. It requires an expensive cloth-shaded field to obtain the moistur econstancy needed for proper development, and gives a comparatively low yield after all this trouble has been taken in its behalf. The variety known as Cuban is somewhat similar to Sumatra and must also be grown under shade. Its somewhat larger, thicker, stronger and better flavored leaf makes it more desirable than Sumatra, but the relatively low yield and expensive equipment results in a much smaller net cash return to the farmer than he ought to have.

Sumatra and Cuban average about twenty leaves each; Havana seed and Broadleaf, which are grown without shade-cloth, have one or two leaves less. The last two are similar to each other. Just as Sumatra and Cuban form one group of tall and relatively small leafed plants, Havana seed and Broadleaf form a second group of short plants having large coarse-grained leaves. The Broadleaf blade is lance-ovate, and drooping, and is thus not well fitted for economical wrapper cutting. On the other hand, the large leaves (reaching 34 inches) when not too thick and coarse, have the finest



BROADLEAF TOBACCO—THE OTHER PARENT OF ROUND TIP

Very different are the characteristics of Broadleaf tobacco leaves as compared to Sumatra leaves shown on the opposite page. The leaves of Broadleaf are large and thick, of coarse texture and strong flavor while Sumatra leaves are small, thin and without flavor but possessing the round tips. The hybrid of the fourth generation from the crossing of these two plants is what is now called Round Tip. (Fig. 2.)

texture and flavor of all wrapper tobaccos. The Havana seed leaf is somewhat less coarse than the Broadleaf and is not quite so pointed at the tip

PRODUCING A NEW TYPE

The situation in the valley, then, has been this: Four varieties have been grown, each is imperfect in itself, but differing from each other in such a way that there was the possibility of combining the best characteristics into a single type of marked excellence. A cooperative plant breeding project was undertaken by the Connecticut Agricultural Experiment Station, Harvard University and the United States Department of Agriculture, therefore, with this object in view.

After examining the possibilities inherent in all four varieties, Sumatra and Broadleaf were selected as the two parents most promising in their qualities. The chief characteristics in which the two varieties differ, and the qualities which it was hoped might be obtained were as follows:

Broadleaf	*Sumatra*	*Prospective type*
large leaf	small leaf	large leaf
narrow tip	round tip	round tip
drooping leaf	upright leaf	upright leaf
leaves close	leaves apart	leaves close
texture coarse	texture fine	texture intermediate
leaves thick	leaves thin	leaves intermediate
flavor good but strong	flavor none	flavor intermediate
yield high	yield low	yield high

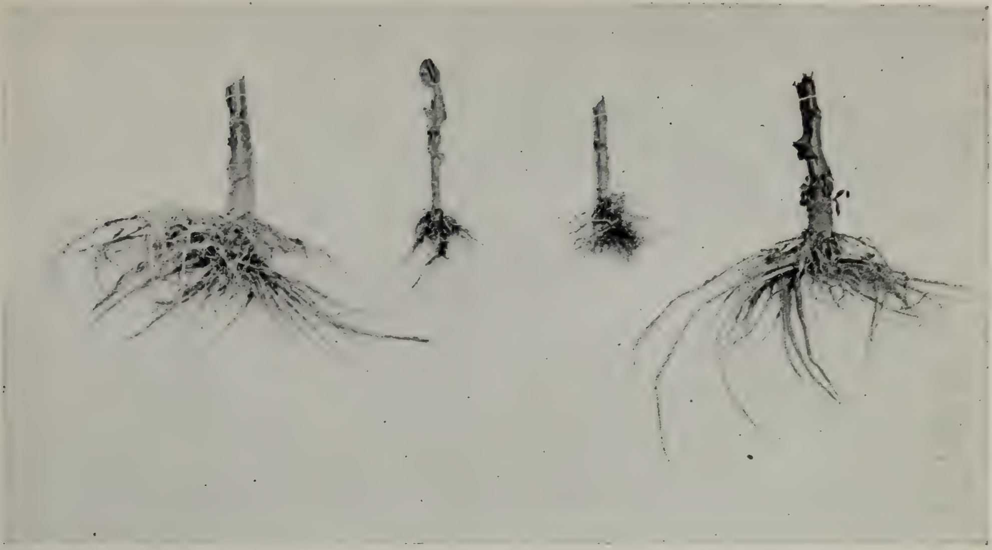
ROUND TIP TOBACCO SHOWING UNIFORMITY OF GROWTH

This new type of tobacco has produced 2,800 pounds per acre under ideal conditions as compared to about 1,500 pounds per acre from types that are commonly grown. It has a greater number of leaves, ovate in shape, thus affording a greater yield of cigar wrappers. (Fig. 3.)

The two varieties were crossed and a small first hybrid generation grown. The plants were uniformly intermediate in type, though perhaps approaching the Sumatra rather than the Broadleaf. They grew very quickly, and apparently did not possess a very desirable root system since a slight windstorm would send them to the ground. The leaves were very coarse-veined and worthless commercially. The second hybrid generation was extremely variable, transcending the grand-parental limits in such characters as leaf numbers. Among the plants of the second hybrid were about twenty with the characteristics desired so far as one could tell from field observation. Selfed seed from these individuals was obtained, and a number of F_3 families tested. Some were variable, some were apparently constant. After careful statistical study in the field, half a dozen selections were carried to the fourth hybrid generation and further eliminations made after the leaves had been sorted and cured. The selection surviving this fine-grained seive was named the Round Tip.

QUALITIES OF THE NEW TYPE

The new tobacco possesses a leaf nearly as large as that of the Broadleaf when grown on fertile soil. Its leaves



SHOWING ROUND TIP'S GREATER RESISTANCE TO ROOT ROT

These are the roots of average plants taken from one field infected with root rot. Those on the outside are Round Tip and those on the inside Havana tobacco. The grower demanded a plant resistant to disease, and one which would stand up under wind and rain—qualities which are prominent in the new tobacco. (Fig. 4.)

ROUND TIP TOBACCO LEAVES

In comparing these leaves of the new hybrid with those of the two parents shown in Figs. 1 and 2, the immense increase in cigar wrapper surface is plainly shown. These are bottom, middle and top leaves from a Round Tip plant. Wrappers made from Round Tip leaves cannot be distinguished from the high grade Sumatra wrappers. (Fig. 5.)

are ovate instead of lanceolate, thus increasing the yield of cigar wrappers. The average leaf number is 26 instead of 19; and the leaves are upright instead of drooping, though they are not grouped together on the stalk as closely as might be desired. The strength, elasticity, grain and color of the cured article is all that could be wished. The lighter colored leaves of a greenish shade are indistinguishable from high grade Sumatra wrappers in appearance, though possessing somewhat better texture and flavor. At a meeting of tobacco growers recently, cigars wrapped with Round Tip could not be separated from cigars wrapped with Sumatra by the most distinguished tobacco experts present. The darker leaves of the brownish grades are very like imported Cuban wrappers, but in the opinion of many have a better burning quality. Crops of 2,800 pounds per acre have been secured, though these were obtained under extraordinarily good conditions. Under average growing conditions yields of about 2,000 pounds per acre may be expected. When it is realized that the types commonly grown yield only about 1,500 pounds, the increase is notable.

It remains to be seen whether a new tobacco of this kind can be introduced to such a conservative market as that to which it must be offered. But the type has now had a three year test, satisfying the requirements of some thirty planters who have grown it on considerable areas. It is as uniform in type as any of the older varieties and possesses, besides the market requirements mentioned above, some field characters which delight the grower. Not the least of these is a wonderful root system making it stand up under winds that lay other tobaccos low, and a resistance to root rot which came to it by accident rather than by design of the plant breeder. One prominent grower says of it: "If it does not largely replace other tobaccos of the Connecticut River district in the near future and add millions of dollars to our wealth, it will be because of timidness on the part of the buyers who have been taught that anything new in the tobacco trade is something to be shunned."

As Genetics Comes of Age

E. M. EAST

Reprinted without change of paging, from the Journal of Heredity (Organ of the American Genetic Association) Vol. XIII, No. 5, Washington, D. C., May, 1922.

AS GENETICS COMES OF AGE

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GENETICS was born twenty-one years ago when there came the first real appreciation of the studies on heredity made in the little garden at Brünn. Now that it has reached full manhood and is ready to assume the *toga virilis*, the time seems fitting to call back the yesterdays, to cast up accounts, and to judge whether the performance of maturity promises to repay the cost of infancy and childhood.

Perhaps it will serve our purpose to contrast the status of affairs toward the close of the long prenatal period previous to the twentieth century, with that of today. When one does this, he is convinced that our chance metaphor is really rather apt. When the chick breaks through the egg, when the butterfly bursts the chrysalis, when the rose bud opens, the change is superficially so revolutionary that one is likely to forget the intensive energy expended in preparation for the natal day. So also with the study of heredity. Genetics was born and christened because of Gregor Mendel. Not because he diligently gathered facts regarding the heredity of the garden pea; rather because he was able to analyze and correlate these facts. Others had gathered facts galore. Indeed the growth-curve of knowledge had been rising steadily for many years. Yet metamorphosis came only when mathematics began to be applied effectively to the efforts of physiologist and morphologist. Change in method rather than a single great discovery gave the first real insight into the master riddle of the ages.

EARLY STUDIES OF HEREDITY

Previous to the beginning of the twentieth century, isolated observations on heredity had been made by many types of workers. It was only natural that this should be the case. Such a seemingly mysterious force could hardly have failed to fascinate mankind from the very beginning of his speculative history. But isolated observations on subjects wherein are numerous complex variables, usually wait long for the keystone with which the generalizing mind can support an edifice of useful theory. And in this particular instance the time was undoubtedly extended by a striking aloofness and lack of a spirit of co-operation among the laborers in the various guilds.

The first tier of foundation stones was laid by the breeder. As was to have been expected, the empiricism of a practical art led the judicial classification and the inductive reasoning of science. One has only to study the wonderful domestic animals in the paintings and reliefs of Babylonia, of Assyria, of Egypt, to realize that knowledge of the effects of selection has been extant for at least six thousand years, perhaps for ten or twenty thousand years. And Jacob's little scheme to mulct his father-in-law of the ring-straked and spotted cattle shows us somewhat of the older theoretical beliefs. Jacob, in fact, seems to have been as advanced a geneticist as many of the animal breeders of the nineteenth century; since the textbooks of this period express a similar belief in maternal impressions and other fables, and contain not a single conclusion that one can now point out as having a permanent value.

Generally speaking, the history of plant breeding gives a little more cause for pride. True, the early Semitic knowledge of plant sexuality was actually lost until the latter part of the seventeenth century; but having rediscovered this fundamental truth through the work of Camerarius, the eighteenth and nineteenth century hybridizers did leave behind them several legacies well worth while. Kölreuter established the fact that reciprocal crosses give very similar results. A little later the efforts of such men as Sageret, Wiegmann, Gärtner, and Naudin, placed three other conclusions on a firm foundation of experiment; the variability of hybrids of the second generation when compared with those of the first, the dominance of certain individual characteristics, and the occasional reappearance of the qualities lost to sight. Possibly analogous observations had been made previously by animal breeders; but it is certainly within the truth to say that even at the beginning of the twentieth century these were not accepted with anything like the unanimity which existed in the botanical field.

Morphology, with a much later start, got down to essentials a great deal more quickly than experimental breeding. Indeed morphologists built so rapidly during the Victorian era they nearly reached a pinnacle of success that would have given us a different day to celebrate. They lacked but the inspiration to put their "ifs" to the test of calm experiment.

THE CHROMOSOMES

Logically it followed from the theory of genetic continuity by cell division that a material substance passed from cell to cell is the basis of all heredity. Naturally, then, the mechanics of cell division was the subject of intense investigation. The result was the discovery that in building up the tissues of the individual organism, in the preparation of the reproductive cells for their special work, and in the behavior of these cells in carrying out that work, there was an essential similarity of the two processes in both animals and plants.

As these studies progressed it became apparent that the cell nucleus was the controlling agent of inheritance, and that within the nucleus the chromosomes played the star rôle. This hypothesis, put forth as a speculation by Haeckel in 1866, within fifteen years gained the support of such eminent investigators as Hertwig, Strasburger and Van Beneden, largely because of the similar elaborate preparations within the nucleus of egg and of sperm during maturation and of their apparently identical contribution of nuclear material in bi-parental inheritance. Numerous investigations on artificial fertilization were made by adherents and opponents of this view; but owing to the experimental difficulties involved, they were not conclusive. Polemic dissertations on the part played by nucleus and cytoplasm followed that were reminiscent of discussions in the realm of religion or of politics. Gradually the proponents of the view gained more and more converts, not because they were able to demonstrate a monopoly of directive action by the nucleus in development and heredity, not because they could prove the intricate organization of so many unfertilized eggs was controlled by nuclear behavior, for such was not the case: it came through small increments to cytological knowledge which gradually wove a mesh so fine that there was no loophole of escape from the conclusion. Belief in the importance of the chromosomes grew, as in the case of organic evolution, not because of direct proof, but because of circumstantial evidence. Without going into an extended argument on the subject, one may recall the constancy of chromosome number in each species, their individuality in size and shape, the exactitude of their division during growth, and their peculiar behavior at the maturation of the germ cells.

NINETEENTH CENTURY THEORIES OF HEREDITY

These facts, together with numerous minor discoveries, were the basis of nineteenth century theories of heredity. But besides the efforts of the practical breeders and of the morphologists, a serious attempt was made by Francis Galton and Karl Pearson to put genetic studies on a firm groundwork of quantitative experiment. Essentially their method was to measure the degree of association between parents and offspring for any particular character. It was wholly a group method, and by its very nature precluded both the analysis of individual cases and the utilization of biological facts among the premises. Its chief generalization, the law of ancestral heredity, wherein the correlation of characters among blood relatives was interpreted as showing the inheritance of an individual to be made up of a series of contributions, one-half from the parents, one-fourth from the grandparents, and so on, has been shown to be erroneous. Having proved no stimulant to productive investigation, its discussion has passed from the genetic literature of today; but the mathematical procedure evolved by the Galtonian school has proved to be extremely helpful.

The earlier genetic theories of the period under consideration necessarily were highly speculative because of the paucity of known facts; but the fundamental postulate of each, active ultramicroscopic living units, has been retained in the genetic theory of today.

Darwin's provisional hypothesis of pangenesis (1868), for example, assumed that such particles, the gemmules, were given off at all times by every cell, and passed to all parts of the body including the germ cells. He thus accounted vaguely for the inheritance of acquired characters and for regeneration of parts, as well as for ordinary heredity.

Among several contemporary modifications of this type of theory was that of De Vries (1889), who assumed that the corpuscles, which he called pangens, represented potential elementary body characters rather than cell qualities, and that the universe of their activity was the cell rather than the body.

It is clear, even with only a glimpse of such theories, that they could satisfy none but the philosophically inclined. They did little or nothing toward stimulating work designed to test the points involved.

A different fate met the speculations of Nägeli (1884). Here was postulated two types of protoplasm built up of physiological units, the micellæ: the one was nutritive in its functions, and required no particular architecture; the other, the idioplasm, a structure of elaborate constitution, was built of units which represented the potential elementary characters of the organism.

WEISMANN'S THEORY

Utilizing this conception, Weismann (1892) evolved a theory which more nearly fulfilled the requirements of an experimental working hypothesis than any of those previously outlined. The idioplasm or germplasm he identified with the chromatin of the nucleus. His ultimate physiological unit, the biophore, was the biological atom active in building up organic characters. Grouped together into higher units, the determinants, these corpuscles controlled the specialization of cells. The various determinants of an organism made up the *ids* contributed by past generations. The *ids*, if more than one, might differ slightly among themselves, thus governing variation within the species. They formed the chromosomes, or *idants*, by arrangement in a linear series.

Denying the inheritance of acquired characters, and doing much toward demolishing the fallacious logic put forth as proof at that time by adherents in the belief, Weismann outlined a very stimulating conception of heredity on this basis. The immortal germplasm was assumed to be set apart at a very early cell division and passed along unchanged to the next generation, except as the activities of the living units produced occasional changes in its constitution. A provision for accurate equational division of the chromosomes and their reduction in number at the maturation of the germ cells was thus demanded, predicted and afterwards realized—though not precisely in the way he supposed—by discoveries in the field of cytology.

Weismann further accounted for evolution by a selective struggle between the determinants of the germ cells, and for individual development by a qualitative distribution of the determinants of those cells set apart to build up the bodies which were to act as hostelries for the immortal germplasm.

With Weismann is reached the peak of genetic generalization at the beginning of the twentieth century. Today we have parted company with him in many particulars, nevertheless if modern genetic theory can be said to be the outgrowth of any earlier school, the Weismannian school must be given the preference. As Wilson has said, he brought "the cell theory and the evolution theory into organic connection." His work, besides dispelling many old wives' notions by its cutting logic, was second only to that of Mendel in making genetics an experimental science. Morgan credits him with "the basis of our present attempt to explain heredity in terms of the cell" in that he propounded three of the principles upon which the modern Chromosome Theory is founded.

WEISMANN AND MENDEL

Some may see an inconsistency in ascribing the ground-work of current ideas of heredity to Weismann, and yet celebrating the rediscovery of Mendel's papers as the true break between the old and the new. The obvious reply would be that it takes more than three foundation stones to prop up a useful structure, and that Mendel furnished several examples, most beautifully cut and polished. But there is a deeper truth than this to be emphasized. Weismann unquestionably had a breadth of mental vision far exceeding that of Mendel. He was a real clairvoyant of science, too, and not a mere visionary in the cynical modern sense of the word. Nor was he above the drudgery of experiment. But he failed to have the good luck of initiating a simple method whereby the elementary quantitative relationship between hereditary phenomena could be tested and retested by those who followed him. This fortune fell to Mendel, who, though in a comparative sense a narrow man, was yet able to grasp somewhat of the significance of the results obtained, and leave an imperishable monument to his name. No one may say he was the greater man, but no one can deny he left the most useful work. His results are a satisfaction to the rank and file of scientists for just this reason. They leave a ray of hope to the plodders with whom most of us trail.

The path opened up by Mendel has joined with the path cleared by morphology to produce a road that has extended some distance during the past two decades; but to point out the cairns and avoid falling into the pits is not an easy task. The roadmakers have been numerous, and in general, honestly constructive; but in order to hold this article within reasonable limits I shall mention few names except to pay a just tribute to Morgan as the master craftsman. Nor shall I speak of the attempts at sabotage except to say they have become more and more infrequent. I shall merely endeavor to recapitulate the fundamental points as best I may with the hope that the effort will not be far afield. At the risk of becoming wearisome I want to try to estimate the progress in terms of general conclusions rather than to describe a heterogeneous selection of ancient heredity puzzles that have yielded to simple interpretations.

GENETICS AND EVOLUTION

First, it must be emphasized that though modern genetics has brought about a clearer orientation of the problems of development and of evolution, it has been concerned directly with the mechanism of heredity. Least progress has been made in connection with the problems of ontogeny. But the conception of where the one ends and the other begins, in so far as this is possible, has become much more definite—at least in this country. It is probable that the interesting phenomena recently described by Mr. Bateson where seeds from various parts of the same plant apparently transmit different characters, would be less likely to appear so puzzling if this were clearly recognized. And even if we admit our inability at present to contribute much toward the solution of the question, so well delimited by Weismann, of somatic specialization during the development of the individual, one cannot but feel that further progress in dealing with problems of straight heredity will ultimately be helpful.

As to the grand problem of evolution, I believe there has been a concrete offering. True, the question of "how" is still in *statu quo;* but one must be rather a pessimist if he does not consider that the current conception of the gene presents something tangible on the subject. It certainly allows a definite distinction between variations due to environmental fluctuations, variations due to rearrangements and combinations of genes, and variations due to change in the constitution of the unit of heredity itself. Furthermore the data now being gathered on the type of gene changes occurring, and on the frequency with which they take place, are not to be cast aside as of no value to the evolutionist. A statement as to just what they mean would be a daring assertion, but that they mean something now and will mean more later cannot be doubted.

Let us take, for example, the following illustrations, which, I think, are fair.

1. Mutations (Gene variations) are now occurring in all species that have been investigated intensively.

2. There is a wide range to mutation frequency in different species.

3. The number of useless or of harmful mutations is *many times the number of useful or of beneficient mutations*.

4. The number of mutations affecting chiefly certain organs or particular tissues greatly exceeds those affecting other parts of the individual.

5. The "conservative" parts as measured by mutation frequency, appear to have slight relation to the "conservative" parts as determined by the circumstantial evidence of the phylogenist. For example, loss of the ligule, a characteristic of the grass family, has been found in a goodly number of the cultivated grasses,—these being the only ones that have been studied very carefully.

6. Mutations are often reversible. Reversibility may not be universal, though the mere fact that it has not been observed in every case proves only that the reaction does not take place in both directions with the same ease.

7. Mutations which from their major effects can be arranged in a graded series,—for example, eye color of *Drosophila melanogaster*,—are found not to have originated in that order. That is to say, such orthogenetic phenomena as have been observed are better interpreted as analogous to chemical phenomena, where tendency to certain reactions is greater than to others, than as "vital force" phenomena.

No one can maintain that these genetic findings compare with the fundamental laws of thermodynamics in elegance and simplicity. No one can say how general they are. But fruit flies and maize, rodents and peas, upon which the observations were largely made, are pretty far apart in the general scheme of things; therefore it would be very odd indeed if they should turn out to be special cases. And to me they are very helpful to a clearer general conception of evolution.

THE MECHANISM OF HEREDITY

Turning now to the mechanism of heredity, let us see what can be said. The main generalization is that there are units of inheritance, the genes, which are constant in the sense that stable chemical compounds are constant; and whose distribution follows the distribution of the chromosomes. In other words, the discoveries of experimental genetics have made it possible to endow the conceptual units of earlier days with particular qualities, just as discoveries in the physical sciences have made it possible to delimit the characteristics of atoms and molecules. Presumably there may be other types of inheritance, but the only one thus far described is an exclusively maternal inheritance of certain plastid characters. And even in this case, it is not absolutely certain we are not dealing with symbiotic organisms that are transferred from host to host in some such way as the numerous more or less yeast-like forms being daily described in relation to insects and other lower animals. The mere fact that numerous dicotyledons and monocotyledons on the one hand, and mammals, birds, amphibians, reptiles, fishes, arthropods, and molluscs on the other, show essentially identical types of heredity, makes it probable that the generalized mechanism has been discovered. At the same time, though the angiosperms, insects, and mammals thus far studied intensively, distribute their units of heredity with a convincing similarity of detail, it is altogether likely that special cases of peculiar distributions will be found later. And there is every reason to believe that these odd or unique types of inheritance will parallel a specialization in chromosome distribution departing somewhat from the one we have come to look upon as regular.

THE LAWS OF HEREDITY

This regular or common scheme of chromosome distribution in sexual reproduction has been the basis of practically all of our present genetic knowledge. From past experience with it one can pick out the following inductions, each of which has been tested with variable degrees of thoroughness. They are not set down here with the idea they are necessarily more inviolable than the laws of the Medes and Persians, but merely with the annotation that no experimental data have thus far overstepped them except those on the plastids. They may be called Provisional Laws of Heredity.

The first five of these generalizations describe the mechanism of heredity in the preparation of the germ cells for fertilization.

1. *There is segregation of paternal genes from maternal genes, each unchanged by the association.* The pedigree culture evidence supplements the cytological evidence in favour of the idea that this segregation takes place at the reduction division of the chromosomes in the maturation of the germ cells, when homologous paternal and maternal genes pair and separate, one of each pair passing to each of the two daughter cells.

2. *There may be any combination of the choice of one out of each pair of genes in making up the genetic constitution of the gametes.*

3. *In transmission to the two daughter cells, certain sets of genes are always manipulated independently of all other genes.* This is a statement of independent inheritance, or rather of independent genetic recombination, without reference to the chromosomes. It is an unworthy piece of quibbling, however, not to accept the simple indication of cytology that this law is the result of the operation of the chromosomes acting as gene carriers. It results from the fact that No. 1 pair of chromosomes, no matter how it may be packed with genes, carts its cargo independently of all other pairs.

4. *The manipulation of genes within a given series is always dependent.* This is the phenomenon of linkage, or association in inheritance. Concretely, we assume that the genes packed within each freight carrier pair, an homologous pair of chromosomes, are mutually dependent to various degrees in their recombinations with each other.

5. *The number of dependent series, or linkage groups is limited.* In the fruit fly, the number corresponds to the number of chromosomes, and the presumption is that this is the case in every species.

Added to these five laws are four more generalizations which refer particularly to the architecture of the germ plasm within the carrier.

6. *There is a stable orderly arrangement of genes.*

7. *This arrangement is linear.* The genes appear to be strung together much as a string of magnetized steel balls.

8. *Rearrangement of genes after linkage breaks is stable, orderly and linear.* By this it is meant to say that rearrangement of the package after interchange of the contents of a pair of carriers is also constant and of the same linear order as in No. 6 and No. 7.

9. *A regularity of behavior has been found in the interference of one crossover or linkage break, with a second rossover in the same carrier, which says that the percent of crossovers varies independently of the per cent of interference.* The converse is also true.

There is a fascination to this picture of germplasm architecture, and the type of investigation which has led to these statements will undoubtedly lead to still greater things in the future; but we should do well to realize that each of these generalizations, except probably the sixth, may be a special case; and that new and different special cases may be found without necessitating a change in the first five conclusions.

There is just one other generalization to be mentioned. It is the only one concerned with fertilization, and upon its truth has depended the discovery of all the others.

10. *There is no selective fertilization between complementary, compatible, functional gametes.*

In other words, if a series of male gametes meets a series of female gametes sufficiently alike to be compatible with them, fertilization takes place by chance. Clearly chaos would result if this were not so. Gametes produced by a single hermaphroditic organism may present hundreds of hereditary differences. The slightest tendency to selective fertilization, therefore, would prevent genetic analysis of the results. Happily, this is not the case. Even in the flowering plants where varying lengths of pistil tissue must be traversed by the male gametophyte before fertilization is possible, proof has been offered that rapidity of passage is not affected by differences in gametic constitution. Genes evidently do not begin to function as such until the life cycle of the new generation commences.

OTHER FACTS OF HEREDITY

There are other categories of facts, several of them probably not flowing out of the above conclusions, that are as interesting to the general biologist as the abstract laws. Without them he cannot get a just idea of the actual concrete working out of the heredity mechanism.

First let us speak of dominance. Dominance was originally defined as an observational phenomenon, the appearance of the effect of one of a pair of different homologous genes, as opposed to the disappearance or exclusion of the effect of the other. Later, it was taken to be the presence of an effect as the antithesis of its absence; and this idea was carried to such lengths that many geneticists came to believe that dominance of "A" over "a" was due to actual absence of any function of "a," or even to the physical absence of any gene whatsoever. Now we have come to see that dominance is a mere arbitrary measurement of the approach of the result "Aa" to that of "AA" or "aa." This has been brought about by finding cases in which the effect "A" or "a" in the haploid condition could be compared with the effects of "AA," or "Aa" and of "aa." These cases make it seem doubtful whether the association of "A" with "a" ever wholly inactivates the latter.

With this conception of the function of the genes in mind it is possible to work out pretty definitely the actual resultant ontogenetic characters after different matings, both with and without linkage, by applying the laws I have just discussed. It is merely a straight mathematical relationship following immediately after acceptance of these basic conclusions. But there are several difficulties involved in identifying the concrete results of breeding with the abstract results of calculation. Some of these difficulties have been leveled, others are yet to be overcome.

One must realize that each gene has many effects on the organism, some of which are not easily discoverable. The fact that a gene is usually ticketed with a name indicative of its most obvious effect on a particular character, should not mislead us in this regard. Then, too, one must remember that many genes affect each organic character; and that similar characters, characters apparently identical, do not necessarily owe their qualities to the same combination of genes. And, finally, it does not follow from any of the above relations, what will be the result of gene interaction. The action of two or more genes may be necessary to bring about a visible or measurable result, though these genes may be carried along separately generation after generation. The full logic of this fact tears down the veil from many an obscure result; since the difficulty of appreciating the results of selection has been due to the failure to realize how many modifying genes may be carried along which have no chance to produce a measurable effect unless a certain basic gene complex necessary for particular organic expression is also present.

This short sketch will show, I hope, that in the twenty-one years of experimental genetics real progress has been made. To be sure, this résumé has been a leaping from crag to crag. No more was possible within the editorial limits here allotted. But if one recalls the scorn of fifty years ago should a daring seer have predicted such a triumph for quantitative mechanical analysis in a subject so overstrewn with variable factors, he in turn will scorn the Freudian over-compensation of today's critic who makes the taunt that it shall go no further.

STERILITY IN WHEAT HYBRIDS

II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS

A THESIS SUBMITTED BY

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TO THE FACULTY OF THE BUSSEY INSTITUTION OF HARVARD UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF SCIENCE
1922

Reprinted from GENETICS 7: 513-552, November, 1922

STERILITY IN WHEAT HYBRIDS. II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS

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Received April 24, 1922

TABLE OF CONTENTS

INTRODUCTION 513
Previous cytological work with wheat 514
Material and methods 516
The chromosomes in the cultivated species of wheat 517
The chromosomes in sterile and partially sterile species hybrids 520
  T. monococcum × *T. turgidum* 520
  Emmer group × Vulgare group 521
The pollen grains in parents and F_1 hybrids 523
Discussion 526
  Polyploidy in plant species 526
  Genetic behavior of polyploid species 527
  Chromosome behavior in certain partially sterile hybrids 530
  Chromosome behavior and sterility 533
  Sterility in wheat hybrids 535
  The relation of chromosome behavior to sterility in wheat hybrids 535
  The origin of tetraploidy and hexaploidy in wheat species 540
  Chromosome number and adaptability in wheat species 541
  The value of tetraploidy 543
  Pollen-grain variability 545
SUMMARY 547
LITERATURE CITED 550

INTRODUCTION

The cultivated species of wheat can be divided into three definite groups according to their sterility relationships in interspecific crosses (SAX 1921). The Einkorn group contains only one species, *Triticum monococcum;* the Emmer group consists of *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum;* the Vulgare group consists of *T. Spelta*, *T. vulgare*, and *T. compactum*. Einkorn crossed with members of the Emmer group or with members of the Vulgare group results in F_1 hybrids which are almost if not quite sterile. Species of the Emmer group crossed with species of the Vulgare group result in partially sterile F_1 hybrids. The species within each group are inter-fertile. These sterility relationships are in accord with the recent taxonomic classifications (TSCHERMAK 1914, PERCIVAL 1921), with serological relationships as determined by ZADE (1914), and with VAVILOV's (1914) classification in respect to rust resistance.

In 1917 a study of the chromosomes of the wheat species and their hybrids was undertaken to determine the chromosome relationships in the cultivated species and the chromosome behavior in partially sterile hybrids. A knowledge of the chromosome behavior should be of value both in an analysis of the origin and relationships of the various species and in a genetic analysis of partially sterile hybrids. A cytological and genetic analysis of wide species crosses involving a certain amount of incompatibility, is especially important in view of EAST's (1921) conclusions in regard to the origin of important domesticated plants and animals.

PREVIOUS CYTOLOGICAL WORK WITH WHEAT

A comparatively large number of investigators have made cytological studies of the chromosome number in wheat. OVERTON (1893), KÖRNICKE (1896), DUDLEY (1908), NAKAO (1911), BALLY (1912, 1919) and PERCIVAL (1921) all report 8 as the haploid chromosome number in *T. vulgare*. SPILLMAN (1912) reports about 6 chromosomes in rye and about 40 in wheat. In all of these cases the papers are very poorly illustrated or are not illustrated at all. PERCIVAL and SPILLMAN present no drawings or figures to support their conclusions. In the other papers the drawings are not convincing, and if they faithfully represent the preparations the material was undoubtedly poorly fixed. The chromosomes are pictured as more or less shapeless masses and lack detailed shape and structure. Such an appearance is often due to poor fixation.

In 1917 the writer (SAX 1918) found about 28 chromosomes in the first division of the fertilized egg cell in *T. durum*. At about the same time SAKAMURA (1918) reported the following chromosome counts in wheat: *T. monococcum*, 14 (diploid); *T. durum*, *T. polonicum*, *T. turgidum* and *T. dicoccum*, 28; and *T. Spelta*, *T. vulgare* and *T. compactum*, 42. SAKAMURA's counts were made almost exclusively from preparations of root tips. The paper contains no illustrations of any kind.

The behavior of the chromosomes in partially sterile wheat hybrids has been described by KIHARA (1919). The hybrids used were *T. durum* × *T. vulgare*, *T. turgidum* × *T. compactum*, and *T. polonicum* × *T. Spelta*. The chromosome numbers of the parents were not determined, but were based on the work of SAKAMURA (1918). The F_1 hybrids were found to have 35 diploid chromosomes, the sum of the gametic number of the parents. The behavior of the F_1 chromosomes in the reduction divisions is described as follows. At the time of the heterotypic division 14 bivalent and 7 univalent chromosomes are formed. The double chromosomes become oriented on the equatorial plate, but some of the single chromosomes may lie outside of the equatorial plate. As the double chromosomes divide normally and pass back to the poles, the single chromosomes become oriented on the plate, divide equationally and join the 14 chromosomes already at either pole. In the homotypic division 14 chromosomes pass to the poles leaving about 7 (often 5 or 6) chromosomes on the plate. These 7 chromosomes do not divide equationally at this time but pass apparently at random to either pole without dividing. Tetrads are usually formed normally but in some cases chromatic bodies are found in the cytoplasm of the one-nucleate microspores. Most of KIHARA's preparations were obtained from anthers, but somatic tissue was also used for determining the chromosome number in the F_1 plants.

KIHARA (1921) has also investigated the chromosome number in the F_2, F_3 and F_4 of the above partially sterile hybrids. In 8 F_2 plants the somatic chromosome number was found to vary from 31 to 42. In 16 F_3 plants the chromosome number varied from 28 to 41, and in 2 F_4 plants the chromosome number was 39 and 42. The number of univalent chromosomes was found to vary from 0 to 5. Some correlation was found in the F_3 between chromosome number and fertility,—the plants with a chromosome number approaching 42 were more fertile than plants with 38 or 39 chromosomes. The paper is illustrated with one plate of microphotographs which, although hardly adequate to support the author's conclusions, do indicate that he had excellent preparations.

It is evident that there is considerable dissension in regard to the chromosome number in wheat. Six investigators report 8 as the haploid chromosome number, one investigator reports about 40, two investigators report 14 for *T. durum*, and one investigator finds 7, 14 and 21 according to the species used. The last investigator (SAKAMURA) does not present any illustrations to support his counts. Since the value of a cytological paper is largely dependent on the character of the illustrations used, it is not surprising that SAKAMURA'S results have been questioned (PERCIVAL 1921). In view of the different chromosome numbers reported by various investigators and the paucity of illustrations presented, a thorough cytological investigation of the chromosomes of wheat, with adequate illustrations, is justified.

MATERIAL AND METHODS

The following species of wheat were used in the present investigation:

Species	*Variety*
Triticum monococcum L.	*Hornemanni* Körn.
Cultural species	
T. durum Desf.	*hordeiforme* Körn. (Kubanka)
(*T. aestivum* L. subsp. *durum* (Desf.) Thell.)	
T. polonicum L.	*villosum* Körn. (Polish)
T. turgidum L.	*pseudo-cervinum* Körn. (Alaska)
(*T. aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *turgidum* (L.) Druce)	
T. vulgare Vill.	*lutescens* Körn. (Marquis)
(*T. sativum* Lam. *T. aestivum* L. subsp. *vulgare* (Vill.) Thell.)	*erythrospermum* Körn. (Preston) *albidum* Körn. (Amby) (Bluestem)
T. compactum Host.	*Humboldtii* Körn. (Wash, Hybrid 143.)
(T. *aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *compactum* (Host.) Sacc.)	
T. *Spelta* L.	
(*T. aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *Spelta* (L.) Sacc.)	*album* Körn.

The chromosome behavior in the following F_1 hybrids was investigated.

(1) *T. monococcum* ♀ × *T. turgidum* ♂
(2) *T. compactum* ♀ × *T. durum* ♂
(3) *T. vulgare* (Amby) ♀ × *T. durum* ♂
(4) *T. durum* ♀ × *T. vulgare* (Amby) ♂
(5) *T. durum* ♀ × *T. vulgare* (Bluestem) ♂
(6) *T. vulgare* (Bluestem) ♀ × *T. durum* ♂
(7) *T. vulgare* (Bluestem) ♀ × *T. turgidum* ♂

For convenience the species of wheat may be divided into the three sterility groups, the Einkorn group, the Emmer (E) group, and the Vulgare (V) group. In measuring pollen grains several additional varieties of *T. vulgare* were used as well as *T. aegilops* Beauv.

The cytological work was started at BUSSEY INSTITUTION in 1917 but was interrupted until 1919 when it was resumed in Illinois. Since 1920 the work has been carried on at the MAINE AGRICULTURAL EXPERIMENT STATION and at the BUSSEY INSTITUTION.

Considerable difficulty was experienced at first in obtaining well fixed material. Often the chromosomes were found clumped together in such a way that accurate counts could not be made. SAKAMURA was unable to obtain well fixed anthers and used root tips for his preparations. Apparently none of the earlier investigators were able to get good fixation of wheat chromosomes at the time of the reduction divisions, if we may judge from the appearance of the illustrations. Poor fixation results in a grouping of the chromosomes into a varying number of shapeless masses of chromatin. I have frequently found approximately 8 such masses in poorly fixed material. In well fixed pollen mother cells the chromosomes are clearly defined, there is little shrinkage, and the cytoplasm shows great detail of structure.

The usual fixatives,—chrom-acetic acid, and Flemming's solution, gave good results in some cases, but the best results were obtained with a modified Bouin's solution developed by ALLEN (1916). This fixative has been used successfully in MCCLUNG's laboratory when chromosome counts were desired. The modified Bouin's solution apparently prevents the clumping of the chromosomes and thus facilitates a detailed examination of the individual chromosomes.

In some cases the entire wheat head was fixed when it was about an inch long, but much better results were obtained when the anthers were dissected out. The best fixation was found where the fixative had to penetrate only a thin layer of tissue.

Sections were cut 10–12μ thick and were stained with Haidenhain's iron-haematoxylin. The drawings were done in ink and in all cases were made from single sections. I am greatly indebted to my wife for much assistance with the drawings, to Professor I. W. BAILEY for doing most of the work in preparing the photographs, and to Doctor EAST for valuable suggestions.

THE CHROMOSOMES IN THE CULTIVATED SPECIES OF WHEAT

The chromosomes in the cultivated species of wheat will be considered in order of their sterility and taxonomic relationships.

In Einkorn, *T. monococcum*, there are clearly 7 pairs of chromosomes in the pollen mother cells. At the time of diakinesis the paired chromosomes can be seen twisted about one another (figure 1). Often delicate fibers can be seen between the two members of a bivalent chromosome and between pairs of chromosomes. The bivalent chromosomes shorten, the nuclear membrane disappears, and the chromosomes become oriented on the equatorial plate. A polar view of the chromosomes at this stage is shown in figure 2. In the heterotypic division the spindle fibers have a subterminal attachment to the chromosomes. The division proceeds regularly and there are no lagging chromosomes (figures 3, 4 and 38, plates 1 and 3). Occasionally a bivalent chromosome is drawn out into a long thread during division, as if the two members of the pair were firmly united at one end and were separated only by considerable tension. As the chromosomes pass back to the poles they become distinctly two-parted (figures 4 and 5). At this stage, the late anaphase, the chromosomes can be most readily counted. The numbers of chromosomes are clearly shown in the polar views of the late anaphase by figures 5 and 37. In the telophase the chromosomes lose their identity as individuals and then pass into the resting stage.

The chromosomes of the second or homoeotypic division are long, much like somatic chromosomes, and are difficult to count. The second division is normal and there are no lagging chromosomes (figure 6). This division takes place at right angles to the heterotypic division and in the same plane so that the resulting microspores of each tetrad are in one plane.

In the species of the Emmer group,—*T. durum*, *T. turgidum*, *T. polonicum* and *T. dicoccum*,—there are 14 gametic chromosomes. In *T. durum* and *T. polonicum* the number has been determined in the pollen mother cells, but in other species of this group the number was determined only approximately from somatic counts.

The 14 pairs of chromosomes in the diakinesis of *T. durum* are shown in figure 7. In most cases the double nature of the bivalents is clearly evident. Often some pairs appear to be longer than others but such differences may be due partly to different stages of contraction. The chromosomes on the equatorial plate are shown in figures 8 (*T. polonicum*) and 9 (*T. durum*) and the anaphase is shown in figure 39. When well fixed the chromosomes can be easily counted at these stages. A side view of the metaphase of the heterotypic division is shown in figure 10. The division is regular and there are no lagging chromosomes at any time (figure 11). The two-parted chromosomes on reaching the poles pass into the resting condition and a cell plate is formed (figure 12). The second division is normal and the four cells of the tetrad are formed in one plane (figure 13).

There are 21 haploid chromosomes in the species of the Vulgare group. The chromosomes in the diakinesis are often grouped around the nucleolus, and owing to the relatively large number it is not easy to count them at this stage. Accurate counts can be obtained from the metaphase of the heterotypic division. A polar view of the metaphase of *T. Spelta* is shown in figure 14 and a similar view of the chromosomes of *T. vulgare* is shown in figures 15 and 40. The subterminal attachment of the spindle fibers together with the anastomosis of certain parts of the bivalent chromosomes results in unusual distortion of the chromosomes at the time of division (figure 16). The free ends of the chromosomes are usually short and thick, while the portions between the fiber attachment and the united ends are often drawn out into thin threads. There are no lagging chromosomes at any time either in the anaphase or telophase (figures 17, 19 and 44). The chromosomes in the late anaphase can be easily counted (figure 18). The chromosomes in the second division are very long and can be counted only with difficulty. The approximate number may be counted in figure 20, which is the metaphase of the second division in *T. vulgare*. The division is normal and the tetrads are formed as in the other groups of species.

There is apparently little difference in the size of the individual chromosomes within or between species. Often several long bivalents can be seen in diakinesis in the Emmer and Vulgare groups, but the number of these varies even in the same individual plant. In the side views of the metaphase certain chromosomes are longer than others, but here again the number of very long chromosomes varies and it is probable that the longer chromosomes have no greater volume than some of the shorter ones. In the figures it appears that the individual chromosomes of Einkorn are larger than the chromosomes of the other species, but such an appearance may be entirely due to differences in fixation and staining. The preparations of Einkorn were stained much more deeply than preparations of the other species.

In general there is some correlation between chromosome number and cell size, as indicated by the size of the pollen mother cells in the different species. Often, however, the cells are cut in such a way that a cross section does not indicate their relative volume. Differences in stages of development as well as differences in fixation may also cause differences in apparent size of cells even in the same species.

The chromosomes of rye have never been pictured, so several figures of rye are presented in figures 41A and 41B. The twisted pairs of chromosomes in diakinesis is especially well shown in figure 41A. In several cases all 7 chromosomes can be counted even at the stage of diakinesis.

THE CHROMOSOMES IN STERILE AND PARTIALLY STERILE SPECIES HYBRIDS

T. monococcum × *T. turgidum*

In the cross of Einkorn × Alaska the F_1 plant has 21 somatic chromosomes, 7 contributed by the Einkorn parent and 14 contributed by the Alaska parent. At the time of the first reduction division about seven pairs of chromosomes are formed, leaving approximately 7 single chromosomes. Presumably the 7 Einkorn chromosomes pair with 7 of the Alaska chromosomes, leaving 7 single or univalent chromosomes contributed by the Alaska parent. The bivalent chromosomes orient themselves on the equatorial plate, but the single chromosomes are usually found at or near the poles of the spindle figure,—seldom on the equatorial plate. In figure 22 there are 6 pairs of chromosomes shown on the heterotypic plate and one pair which has divided. Four single chromosomes lie at one pole and three at the other, a total of 7 single chromosomes. The number of single chromosomes at the poles varies somewhat, but the total number is approximately 7 in most cases (table 1).

TABLE 1

Assortment of single chromosomes in the heterotypic division of the F_1 Einkorn × Alaska.

Number of single chromosomes at either pole	4 3	4 4	5 3	4 2	5 2	6 1	5 4	3 2	6 3	5 6
Total singles	7	8	8	6	7	7	9	5	9	11
Actual frequency	8	4	4	3	3	3	2	1	1	1
Theoretical frequency	17				10	3				

The number of single chromosomes may vary due to segmentation, or they may lie on the plate with the double chromosomes in some cases. It is also possible that less than 7 bivalent chromosomes are formed, in which case the total number of singles would be 9 or 11. In general, however, the chromosome number is approximately 7 and the distribution of about equal numbers of single chromosomes to either pole occurs with greatest frequency as expected.

The single chromosomes remain at the poles while the bivalent chromosomes divide and join the single chromosomes. The total number of chromosomes at either pole is then usually about 10 or 11 (fig. 23). As the members of the bivalent chromosomes pass to the pole they split longitudinally as usual (figure 24). All chromosomes both single and double are grouped together in the telophase (figure 25).

In the homoeotypic division approximately ten chromosomes are found at the metaphase (figure 27). These chromosomes are long and curved. They consist of the 7 divided bivalents plus 0 to 7, but usually 2 to 5, univalent chromosomes. Since the univalents did not divide during the heterotypic division they would be expected to divide normally in the homoeotypic division. The actual behavior of the chromosomes during the second division is difficult to determine owing to the length of the chromosomes and the paucity of critical stages. The division appears to be normal, however, and seldom were any lagging chromosomes found (figure 26). The tetrads are formed as in the case of the parental species and in comparatively few cases are chromatin granules found outside of the nuclei. The tetrads separate into one-nucleate pollen grains which appear to be normal. Very few of the one-nucleate pollen grains develop normally, however, and only about 2 to 3 percent of the mature pollen grains appear normal. Probably none of them is functional.

In a few cases the univalents were found on the heterotypic plate after the bivalents had divided, indicating that the behavior of the univalents in the reduction division may vary in different individuals.

Emmer group × *Vulgare group*

In crosses between members of the Emmer group and members of the Vulgare group the sum of the gametic numbers of chromosomes is 35. Such F_1 plants will have 14 chromosomes contributed by one parent and 21 chromosomes contributed by the other parent. In the pairing of the chromosomes for reduction the 14 chromosomes of the Emmer group presumably pair with 14 chromosomes of the Vulgare group, leaving 7 univalent Vulgare chromosomes. At any rate in diakinesis there are about 14 bivalents and 7 univalents. The bivalent and univalent chromosomes can easily be distinguished in the metaphase of the heterotypic division. A polar view of the metaphase of the F_1 of Amby (V) × Kubanka (E) is shown in figures 28 and 42. The 7 univalent chromosomes are long, slender and V-shaped, while the bivalent chromosomes are like the bivalents in the normal heterotypic division in the parent species. A similar stage is shown in the F_1 of Bluestem (V) × Alaska (E) in figure 29. The univalents are usually arranged on one side of or around the group of bivalents.

Some of the single chromosomes often lie outside of the equatorial plate and appear to be distributed more or less at random in the division figure (figure 30). Usually only two or three single chromosomes can be seen outside of the equatorial plate in a side view, but in a polar view all of the univalents may lie outside of the group of bivalents. As the bivalents divide and pass to the poles the univalent chromosomes become oriented on the equatorial plate (figures 31-34, 43 and 45). The univalents may vary in number due possibly to the failure of all 14 chromosomes to pair, or to the premature or accidental passage of single chromosomes to the poles (figure 30).

TABLE 2

Number of lagging single chromosomes in the heterotypic division of F_1 of Emmer group × Vulgare group.

Number of lagging chromosomes	4	5	6	7	8	9
Frequency	1	3	7	22	2	1

Table 2 was prepared from well fixed division figures before all of the univalents had separated and where the figure was apparently intact. In the majority of cases 7 lagging univalents were found (figures 32 and 34). In one case 9 single chromosomes appeared to be present (figure 31), but at least one univalent may be segmented into two parts. The univalents are frequently somewhat constricted in the centers where the spindle fibers are attached. The grouping of the bivalents on one side of the spindle is often found (figure 33). The 14 chromosomes at the poles, resulting from division of the bivalents, can frequently be counted with little difficulty, but it is difficult to picture them because they are so compactly grouped.

The segmented univalent chromosomes divide and pass to the poles (figure 35). In most, if not in all cases, there are no chromosomes left free in the cytoplasm, but all are grouped together in the telophase (figures 36 and 46). The daughter nuclei thus formed pass into the resting stage.

In the second division some univalent chromosomes are found outside of the equatorial plate in the metaphase (figure 47). In the late anaphase or telophase univalent chromosomes are found lagging after most of the chromosomes have reached the poles (figures 37 and 48). The number of lagging chromosomes varies, with the mode at 5. Perhaps some of the chromosomes at the poles in the metaphase (figure 47) remain there and fail to take their place on the plate, or single chromosomes may accidentally pass to the poles with the original members of the bivalents. The following table shows the distribution of the single lagging chromosomes in the second division.

TABLE 3

Number of lagging chromosomes in the homoeotypic division. Vulgare group × Emmer group.

Number of lagging chromosomes	2	3	4	5	6	7
Frequency	1	3	5	10	5	2

The lagging chromosomes do not divide equationally but pass apparently at random to either pole. Thus the four microspores each contain 14 chromosomes presumably contributed by both parents according to chance and from 0 to 7 additional chromosomes of the Vulgare parent, the latter number also depending on chance distribution. The fact that the univalent chromosomes divide but once, equationally of course, while the bivalents divide twice in the course of gametogenesis, is in accord with the usual conception of the nature of the reduction divisions.

In some cases the lagging chromosomes fail to go back to the poles and several chromosomes may lie outside of the reconstructed nucleus, usually in the vicinity of the newly formed cell wall. As a rule, however, microspores are formed which have no chromatic material in the cytoplasm outside of the nucleus. These one-nucleate pollen grains appear to be entirely normal so far as the morphological structure is concerned. In many cases, however, they are unable to develop into normal pollen grains. About 20 percent of the pollen of the F_1 plants resulting from Emmer group × Vulgare group crosses is obviously imperfect as indicated by smaller size and meager contents. Undoubtedly a larger percentage is not functional because of physiological or other imperfections in pollen grains that appear morphologically perfect.

THE POLLEN GRAINS IN PARENTS AND F_1 HYBRIDS

There is a striking correlation between chromosome number and the size of the pollen grains in the wheat species studied. Such a relation would be expected if BOVERI'S conclusions concerning chromosome number and cell size have a general application. The pollen grains are especially good for testing such relationships because they consist of only several cells, their volumes are easily determined, and they can be meas-

TABLE 4

Size and variability of pollen grains of wheat varieties and species and of some F_1 hybrids. 1921 data.

VARIETY OR F_1 HYBRID		DIAMETER IN MICRONS (CLASS CENTERS)																					N	MEAN	σ	C. V.	VOLUME CU. μ
		37.5	39.0	40.5	42.0	43.5	45.0	46.5	48.0	49.5	51.0	52.5	54.0	55.0	57.0	58.5	60.0	61.5	63.0	64.5	66.0	67.5					
Aegilops ovata	W		1	8	15	15	16	5	2	1													63	43.55 ± .18	2.12 ± .13	4.87 ± .29	53,000
	L								1	3	5	19	20	9	5	1							63	53.55 ± .15	1.80 ± .11	3.35 ± .20	
Einkorn.			3	12	23	33	44	13	6	4													138	44.02 ± .13	2.19 ± .09	4.98 ± .20	45,000
Kubanka	E						1	3	13	19	20	22	10	4	3	1							96	51.22 ± .18	2.62 ± .13	5.12 ± .24	70,000
Alaska	E						2	4	8	13	14	23	22	7	1	1							95	51.76 ± .19	2.68 ± .13	5.18 ± .25	73,000
Polish	E					1	5	6	19	17	27	31	5	1	1								113	50.34 ± .16	2.47 ± .11	4.90 ± .22	67,000
Emmer	E						2	2	8	19	29	26	11	9	4	2							112	51.70 ± .17	2.62 ± .12	5.07 ± .23	72,000
Spelt	V								2	2	4	13	15	17	11	12	8	3		1			88	55.59 ± .23	3.15 ± .16	5.67 ± .28	90,000
Marquis	V									2	5	18	20	27	18	5	4						99	54.91 ± .15	2.27 ± .11	4.14 ± .20	87,000
Amby	V									1	5	14	16	26	15	8	4	2					91	55.30 ± .17	2.47 ± .12	4.46 ± .22	89,000
Preston	V									2	4	21	23	25	20	5	5						105	54.92 ± .15	2.29 ± .11	4.17 ± .19	87,000
Royalton White	V									1	5	15	24	33	14	7	2						101	54.92 ± .14	2.06 ± .10	3.75 ± .14	87,000
Kota V × Royalton	V							1	1	3	7	12	17	21	18	8	4		1				93	54.95 ± .20	2.85 ± .14	5.19 ± .25	87,000
Spelt V × Marquis	V			1	1	1	2	1	6	4	8	9	12	18	17	13	16	5	4	1			119	55.37 ± .29	4.67 ± .20	8.44 ± .37	89,000
Polish E × Emmer	E				1	3	2	7	8	10	13	18	15	12	9	1	1		1				101	51.94 ± .26	3.85 ± .19	7.40 ± .35	73,000
Emmer E × Polish	E			1	1	2	2	4	9	9	9	24	12	10	10	5	3	2	2				105	52.73 ± .29	4.37 ± .20	8.28 ± .39	77,000
Kubanka E×Marquis	V	1	2	3	4	8	8	4	4	3	7	15	10	6	7	11	12	6	4	4	1		120	53.04 ± .43	6.39 ± .30	13.07 ± .58	78,000

ured at the same stage of development. The size and variability of the pollen grains of most of the wheat species and of F_1 hybrids between varieties, compatible species, and members of different sterility groups are shown in table 4.

In *T. aegilops ovata* the pollen grains were not spherical and two dimensions were taken to determine the volume. In all other cases the pollen grains were approximately spherical, so only the diameter was measured. In all cases only the apparently good pollen grains were used. Only in the partially sterile hybrid Kubanka × Marquis, was the percentage of obviously poor pollen grains more than 1 to 2 percent. The pollen grains were mounted in lactic acid and were measured with an ocular micrometer.

In Einkorn with 7 chromosomes the mean volume of the pollen grains, measured in thousands of cubic microns, is 45. In the species of the Emmer group with 14 chromosomes the mean volume of the pollen grains is about 70. In the species of the Vulgare group the mean volume of the pollen grains is about 88. The F_1 hybrids resulting from crossing members of the Vulgare group, have pollen grains of about the same size as the parents, and a similar condition obtains for F_1 hybrids within the Emmer group.

Not only is there a consistent and significant relation between pollen-grain size and chromosome number in the various species groups, but there is also a consistent relation between heterozygosis and pollen-grain variability. In the homozygous parents the coefficient of variability for pollen-grain diameter ranges from $3.75 \pm .14$ to $5.67 \pm .28$. In the cross, Kota × Royalton White, both parents belong to the same species, *T. vulgare*, and the pollen-grain variability is little if any greater than for the parents. In the cross, Spelt × Marquis, the parents belong to different species, but to the same sterility group, and the pollen of the F_1 is significantly more variable than for the parents or the preceding varietal cross. The reciprocal crosses between Polish and Emmer also involve different species, but only one sterility group, and the pollen-grain variability in the F_1 is significantly greater than in the parents. The F_1 resulting from Kubanka × Marquis has pollen grains almost three times as variable as the parent species. In this case the parents belong to different sterility groups and the F_1 is partially sterile. The striking increase in pollen-grain variability is not due to obviously aborted pollen grains because only the apparently good pollen grains were measured It will be noted that the distribution is not especially skew or bimodal as would be the case if the increased variability were due only to poorly developed or aborted pollen.

Apparently environmental conditions may cause differences in pollen-grain size. The size of the pollen grains in 1920, measured in thousands of cubic microns, was 72 for Einkorn, 94 for the Emmer group and 114 for the Vulgare group. The relative sizes of the pollen grains of the three sterility groups was about the same in 1920 and 1921.

Pollen-grain size and chromosome number are also correlated in a similar manner in the species of oats.

DISCUSSION

Polyploidy in plant species

The occurrence of the gametic chromosomes in multiples of 7 in the species of wheat suggests that the Emmer and the Vulgare groups are tetraploid and hexaploid forms, respectively, of an original type with 7 chromosomes. The origin of new species by tetraploidy in other genera would tend to support such a view. *Oenothera gigas*, a mutant from *Oe. Lamarckiana*, is the best known case of tetraploidy in plants. The chromosome number of the mutant was found to be 28 as compared with 14 for the parental species (LUTZ 1907). Miss DIGBY (1912) found a tetraploid form of *Primula kewensis* with 36 somatic chromosomes. Tetraploidy has been found recently in Datura (BLAKESLEE, BELLING AND FARNHAM 1920). In all of the above cases tetraploidy has occurred while the species were under observation. The hereditary behavior of the tetraploid plants shows a doubling of genetic factors and thus supports the cytological observations.

Chromosome duplication has also been found in other species which have not been under such close observation and which have not been tested by breeding experiments. In Hieracium there are species with 18, 27, 36 and 42 somatic chromosomes (ROSENBERG 1917). In Crepis species are found with 6, 8, 10, 16, 18, 24 and 42 somatic chromosomes. ROSENBERG (1920) believes that the species with the higher chromosome numbers are caused by the duplication of one or more pairs of the chromosomes in the primary species, *C. virens*, with 3 pairs of chromosomes. In the species with 8 chromosomes the medium-sized pair of chromosomes is reduplicated while in the "*gigas*-mutant," *C. Reuteriana*, each pair is reduplicated. In the genus Rosa species are found with 14, 21, 28, 35 and 42 somatic chromosomes (TÄCKHOLM 1920).

In Chrysanthemum TAHARA (1915) finds in different species haploid numbers of 9, 18, 27, 36 and 45. GATES (1913) lists a number of other cases of tetraploidy in plant and animal species and additional cases have been found in the past few years.

Genetic behavior of polyploid species

In tetraploid plants the doubling of the chromosome number results in a duplication of the genetic factors. Thus tetraploidy can be detected not only by chromosome counts but also by the genetic behavior of hybrids of tetraploid races. If the reduplicated chromosomes of a tetraploid or hexaploid species, or to use BLAKESLEE'S (1920) terminology, the members of the tetrasomic and hexasomic sets, assort in pairs, the usual 15 : 1 or 63 : 1 ratios will result in F_2. If, however, the chromosomes of a tetrasomic or hexasomic set assort at random in an F_1 hybrid a 35 : 1 or 399 : 1 ratio will be found in the F_2. Furthermore, in such tetraploid and hexaploid plants the number of recessive factors may often exceed the number of dominant factors in heterozygous individuals in F_2 hybrids. There is evidence that in some cases either allelomorph of a pair of characters may be dominant if an excess of factors of one character are present. In the case of starchy and corneous endosperm in maize, HAYES and EAST (1915) have found that the character borne by the mother plant was always dominant, due presumably to the dominance of the two factors contributed by the mother, as a result of polar fusion, over the single factor contributed by the male parent. In Drosophila it has been found that the two recessive genes for vermilion and sable dominate the normal allelomorphs. When the mutant and normal factors are in equal numbers the normal is dominant (MORGAN 1919). Sex in Drosophila is determined by the ratio of X chromosomes to autosomes. BRIDGES (1921) has found that the ratio of 2 X : 2 sets of autosomes produces a female while 1 X : 2 sets of autosomes produces a male and the ratio 2 X : 3 sets of autosomes produces an intermediate condition, the intersex. There is also evidence that characters other than sex are also influenced by chromosome ratios.

It is possible that an excess of recessive factors may be dominant over the normally dominant factors in many cases, but because such cases become evident in diploid species only in endosperm characters, or in case of non-disjunction or other unusual chromosome behavior, they are seldom detected. The dominance of the normally recessive character would never occur in diploid plants except in endosperm characters, but in triploid, tetraploid and hexaploid plants such a relationship of dominant and recessive characters would be found. Although few such cases have been discovered it is a possibility to be considered. We have then two possible causes for unusual genetic ratios in polyploid plants,—random assortment of chromosomes and reversed dominance due to an excess of recessive factors. The gametic constitution and genetic ratios for various combinations of diploid, tetraploid, and hexaploid hybrids are given in table 5.

In case of random assortment of chromosomes in a tetrasomic set the F_2 ratio becomes 35 : 1 instead of 15 : 1 which is found if the chromosomes assorted in pairs. In the hexasomic set random assortment would result in an F_2 ratio of 399 : 1 and in most F_2 plant populations such a segregation would not be detected. It is also obvious that a tetraploid individual can never be homozygous for a single factor in case of random chromosome assortment and in hexaploid individuals neither one nor two factors can exist in the homozygous condition. If, then, a mutation occurs in a single chromosome of a hexasomic set in which the chromosomes assort at random the homozygous condition will be attained only when all 3 chromosomes of the set are either dominant or recessive. In a homozygous hexaploid race one- and two-factor differences will not be found with random assortment of the members of the hexasomic sets.

An examination of the hereditary behavior of tetraploid species indicates that in most cases studied the chromosomes assort at random in the tetrasomic sets. MULLER (1914) in analyzing GREGORY'S data on tetraploid Primulas concluded that where more than 2 factors are present, which are normally allelomorphic to each other, the pairing of these allelomorphs usually takes place at random. He interpreted the ratio of 4.5 : 1 in a back-cross as a 5 : 1 ratio expected, rather than a 3 : 1 ratio, and showed that the ratio of 119 : 4 in F_2 may be interpreted as a 35 : 1 ratio rather than a 15 : 1 ratio proposed by GREGORY. MULLER'S analysis of GREGORY'S data indicates that in the tetraploid Primula the chromosomes of a tetrasomic pair assort at random. In Datura the duplicated chromosomes of the Poinsettia mutant apparently assort at random according to BLAKESLEE, BELLING AND FARNHAM (1920). Unusual ratios are obtained because the Poinsettia character is not carried by the pollen to any significant extent. In *Oenothera gigas* certain races when selfed produce 1 to 2 percent *nanella* mutants and pedigrees are found approaching a 3 : 1 ratio. BLAKESLEE'S (1921) analysis of DE VRIES'S data indicates that the 1 to 2 percent *nanella* segregates are recessive in a 35 : 1 ratio, and the fact that dominants from a 3 : 1 ratio throw 3 : 1 ratios or 35 : 1 ratios supports BLAKESLEE'S

TABLE 5

Result of crossing diploid, tetraploid, and hexaploid species when (1) the allelomorphic chromosomes unite in homologous pairs, and when (2) they pair at random. The F_2 *ratios, R : r, are given when (a) an Rrr genotype appears as an R phenotype and when (b) an Rrr genotype appears as an r phenotype.*

		CHROMOSOMES UNITE IN HOMOLOGOUS PAIRS			CHROMOSOMES OF POLYSOMIC SET PAIR AT RANDOM		
Parents	F_1	F_1 gametes	(a)	(b)	F_1 gametes	(a)	(b)
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1\\r_1\end{matrix}$	R_1R_2 r_1	$R_1R_2\text{-}r_1\text{-}R_2\text{-}r_1R_2$	15 : 1	13 : 3	$R_1R_2\text{-}R_1r_1\text{-}r_1R_2\text{-}r_1\text{-}R_1\text{-}R_2$	35 : 1	31 : 5
$\begin{matrix}R_1\\R_1\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	R_1 r_1r_2	$R_1\text{-}r_1r_2\text{-}R_1r_2\text{-}r_1$	3 : 1	3 : 5	$r_1r_2\text{-}r_1R_1\text{-}r_2R_1\text{-}R_1\text{-}r_1\text{-}r_2$	3 : 1	13 : 23
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	R_1R_2 r_1r_2	$R_1R_2\text{-}R_1r_2\text{-}r_1R_2\text{-}r_1r_2$	15 : 1	11 : 5	$R_1R_2\text{-}R_1r_2\text{-}r_1R_2\text{-}r_1r_2\text{-}R_1r_1\text{-}r_1R_2$	35 : 1	3 : 1
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1R_2R_3$ $r_1r_2r_3$	$\left\{\begin{matrix}R_1R_2R_3\text{-}r_1R_2R_3\text{-}R_1r_2R_3\\R_1R_2r_3\text{-}r_1r_2r_3\text{-}R_1r_2r_3\\r_1R_2r_3\text{-}r_1r_2R_3\end{matrix}\right\}$	63 : 1	42 : 22	$\left\{\begin{matrix}R_1R_2R_3\text{-}r_1R_2R_3\text{-}R_1r_2R_3\text{-}R_1R_2r_3\\r_1r_2r_3\text{-}R_1r_2r_3\text{-}r_1R_2r_3\text{-}r_1r_2R_3\\R_1R_2r_2\text{-}r_1R_3r_3\text{-}r_1R_2r_2\text{-}R_1R_3r_3\\R_2R_1r_1\text{-}r_2R_3r_3\text{-}r_2R_1r_1\text{-}R_2R_3r_3\\R_3R_1r_1\text{-}r_3R_2r_2\text{-}r_3R_1r_1\text{-}R_3R_2r_2\end{matrix}\right\}$	399 : 1	282 : 118
$\begin{matrix}R_1R_2r_3\\R_1R_2r_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1R_2r_3$ $r_1r_2r_3$		15 : 1	22: 42		24 : 1	2 : 3
$\begin{matrix}R_1r_2r_3\\R_1r_2r_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1r_2r_3$ $r_1r_2r_3$		3 : 1	0 : 64		3 : 1	0 : 400
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}R_1r_2r_3\\R_1r_2r_3\end{matrix}$	$R_1R_2R_3$ $R_1r_2r_3$		64 : 0	15 : 1		400 : 0	24 : 1
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	$R_1R_2R_3$ r_1r_2		63 : 1	3 : 1		399 : 1	324 : 76
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	R_1R_2 $r_1r_2r_3$		15 : 1	26 : 38		3 : 1	1 : 24

conclusions. Blakeslee suggests that all of the Oenothera mutants are caused either by chromosomal duplication or are due to crossovers from balanced lethals as suggested by Muller (1918). In connection with Muller's hypothesis, however, it may be suggested that if we accept the telosynaptic method of reduction described in Oenothera by Davis (1911) and Gates (1915) and at the same time accept the mechanical chiasmatype hypothesis of crossing over, there would be little or no opportunity for crossing over to occur.

In the case of Oenothera, Primula and Datura, the tetraploid forms have originated in experimental cultures and in all cases the chromosomes of a tetrasomic set appear to assort at random. In wheat there is no evidence that random assortment of chromosomes takes place in tetrasomic and hexasomic sets. The results of Nilsson-Ehle (1909), the Howards (1912) and Gaines (1917) all show ratios closely approximating 15 : 1 or 63 : 1 in wheat hybrids involving several independent similar, or possibly identical, factors for the same character. Segregates can be obtained from the F_2 of a trihybrid, which are homozygous for 1 or for 2 factors, an impossible result in case of random chromosome assortment. In the segregation of capsule form in Bursa as described by Shull (1914) the F_2 ratio approximately 24 : 1 might suggest a random assortment of chromosomes in a hexasomic set with 4 dominant factors, but such a parental type would not exist in a homozygous condition and the behavior of the F_2 segregates proves that 2 factors are involved which assort in pairs. The fact that a character may be determined by several factors does not necessarily indicate that these factors are the result of chromosome duplication.

In both wheat and Bursa multiple factors, presumably allelomorphic and in duplicate chromosomes, do not assort at random as they do in case of tetraploid Oenotheras, Primulas and Daturas. The difference in chromosome behavior may be due to the recent origin of tetraploidy in some species as compared with wheat. It is also possible that the increased chromosome number is not due to duplication of the fundamental chromosome number, or if it is, the individual chromosomes may have changed so that they assort in pairs.

Chromosome behavior in certain partially sterile hybrids

Hybrids between diploid and tetraploid, and between tetraploid and hexaploid, wheat species are completely or partially sterile, and irregular chromosome behavior occurs in the reduction divisions. In the F_1 hybrids between Einkorn with 14 chromosomes and a member of the Emmer group with 28 chromosomes there are 7 bivalents and 7 univalents in the heterotypic spindle. The bivalents divide normally in both meiotic divisions while the singles pass at random, without dividing, to either pole in the first division and presumably divide in the second division. In hybrids between members of the Emmer group with 14 chromosomes with members of the Vulgare group with 21 chromosomes the 14 pairs of chromosomes divide as usual in meiosis while the 7 univalents lag behind but ultimately divide equationally in the first division and pass at random, without dividing, to either pole in the homoeotypic division. A comparison of chromosome behavior and sterility in similar hybrids in other genera is of interest.

In Drosera (Rosenberg 1909) the chromosomes of the F_1 of a cross between a species with 10 haploid chromosomes and a species with 20 chromosomes, behave like the chromosomes in the Einkorn × Alaska cross in wheat. In the reduction division 10 bivalents and 10 univalents are found. The single chromosomes lag and apparently go at random to either pole without dividing. The second reduction division is not described.

In the F_1 of *Oenothera lata* × *Oe. gigas* Gates (1909) found the chromosome number to be 20 or 21, the sum of the gametic numbers of the parents. In the reduction division 10 or 11 paired chromosomes were found on the heterotypic spindle. The division is somewhat irregular in some cases, but Davis (1911) has found a rather loose association of the chromosomes in the parental types. Geerts (1911), however, found 7 bivalents and 7 univalents in the reduction division of the F_1 of *Oe. Lamarckiana* × *Oe. gigas*. The single chromosomes pass to either pole without dividing, usually 3 to one pole and 4 to the other. In the second division the univalents also divide irregularly.

The number of somatic chromosomes in species of Hieracium ranges from 18 to 42. In *H. excellens* (Rosenberg 1917) with 42 chromosomes, 18 pairs of chromosomes are found on the heterotypic spindle in addition to 6 univalents. In the F_1 of *H. auricula* (18 chromosomes) × *H. aurantiacum* (36 somatic chromosomes), 9 bivalents were found with 8 or 9 univalents in the reduction division. The univalents divide irregularly. Similar chromosome behavior was found in other hybrids but often the number of univalents was found to vary even in the same cross.

Täckholm (1920) has found only paired chromosomes in the series 7, 14 and 21, in some Rosa species. Other species, especially those of the Canina section, have both bivalents and univalents at the time of reduction, usually 7 bivalents with 14, 21 or 28 univalents. The species which have both bivalents and univalents in pollen reduction are thought to be hybrids which have been perpetuated for long periods of time by apomictical reproduction.

The behavior of the chromosomes in reduction divisions of species of Rosa has also been described in considerable detail by BLACKBURN and HARRISON (1921). In *R. Sabini* (*R. pimpinellifolia* × *R. sylvestris*) with 42 chromosomes, there are 14 bivalents and 14 univalents on the heterotypic spindle. The univalents lag behind in division but ultimately divide and pass to the poles. In the second reduction division the original bivalents pass rapidly to the poles and the daughter nuclei are reconstructed before the original singles reach the poles. Thus the major nuclei contain about 14 chromosomes. The univalent chromosomes form micronuclei and often a total of 8 nuclei are found which may be termed an "octad."

In one case in the above hybrid all of the chromosomes at the time of the homotypic division were on a single spindle. If development had been allowed to proceed the gametes would be able to produce a new plant octoploid in chromosome number.

In other Rosa hybrids BLACKBURN and HARRISON find a type of reduction similar to that found in *R. Sabini*, but involving 7 bivalents and 21 univalents or 14 bivalents and 21 univalents, etc. All roses showing such partial reduction are facultatively apomictical, due, according to the authors, to the stimulus of heterozygosis.

In Canna and Datura (BELLING 1921) the chromosome behavior in triploid individuals is quite different from that found in similar cases in Triticum, Rosa, Drosera, and Hieracium. In one clone of Canna the 27 chromosomes unite into 9 triads at reduction. The chromosomes of each triad pass at random, two to one pole and one to the other.

Among species hybrids in animals the chromosome behavior in F_1 reduction may vary greatly. FEDERLEY (1915) crossed two species of butterflies, each with 28 chromosomes. In the F_1 the chromosome number varied from 28 to 33 in spermatogensis, due to the varying number of single and double chromosomes on the plate. FEDERLEY believes that there is a negative correlation between the number of paired chromosomes and sterility in such cases. In another species cross FEDERLEY (1916) found little or no failure of the chromosomes to pair in reduction. In the F_1 of *Pygaera curtula* L. (29 chromosomes) with *P. anachoreta* F. (30 chromosomes) FEDERLEY (1913) found about 59 chromosomes in the reduction division, indicating that little or no pairing of chromosomes occurred. In a back-cross of the F_1 with *P. anachoreta* the chromosomes of the same species paired leaving 29 single *curtula* chromosomes. The univalents divided equationally in both divisions, but the second division was often abnormal.

In general, triploid hybrids derived from species with different chromosome numbers have a similar type of chromosome behavior in the reduction divisions. Usually the number of bivalents is equal to the gametic number of chromosomes found in the parent with the smaller number, and the number of univalents is the difference between the chromosome numbers of the parental species. The bivalents divide normally while the univalents usually divide but once, equationally of course, and pass at random to either pole without dividing in one of the reduction divisions. Whether the single chromosomes divide in the first or second reduction division should make but little difference so far as the genetic results are concerned, providing that they divide but once. In case some or most of the univalents are not included in the functional pollen grains, as found in Rosa, the genetic behavior will be disturbed.

Chromosome behavior and sterility

The occurrence of a triploid chromosome number or the reduplication of a single chromosome is associated with more or less sterility. The relation of chromosome number and behavior to sterility is shown for Oenothera, Datura, and Triticum in table 6.

The percentage of apparently poor pollen grains as an indication of sterility is, of course, only an approximate measure, but for general purposes it is considered satisfactory. In Oenothera the species and hybrids having an additional chromosome or those which are triploid are much more sterile than the diploid or tetraploid types. Here, of course, the sterility may be disturbed also by the hybrid nature of most of the Oenotheras or due to balanced lethal factors. In Datura the reduplication of but a single chromosome results in some sterility, still greater sterility is found in the triploid individual, while the tetraploid species is as fertile as the diploid parental type. Tetraploid and hexaploid species of wheat are as fertile as diploid species. In pentaploid hybrids with 14 bivalents and 7 univalents the sterility is much less than in triploid hybrids with 7 bivalents and 7 univalents. The degree of sterility may, however, be greater than indicated by the appearance of the pollen grains. Sterility based on grains set per spikelet shows total sterility for the triploid hybrid and somewhat greater than 50 percent sterility in the pentaploid hybrid as compared with the parents. If we take into account the effect of hybrid vigor and compare the F_1 sterility

Table 6

Relation between chromosome number and sterility.

SPECIES	CHROMOSOME NUMBER	AVERAGE PER-CENT OF POOR POLLEN
Oenothera (Gates 1915)		
Oe. gigas	28	54–72
Oe. Lamarckiana	14	42
Oe. lata rubricalyx × *rubricalyx* segregate	14	22
Oe. lata rubricalyx	14	33
Oe. semilata	15	77
Oe. lata-like	15	76
Oe. rubricalyx × *gigas*	21	81
Oe. gigas × *lata rubricalyx*	22	89
Datura (Blakeslee 1921)		
Normal	24	3
"New species"	48	3
Triploid	36	34
Trisomic (12 mutants)	25	8–21
Triticum (Sax 1921)		
Vulgare group	42	1
Emmer group	28	2
Einkorn group	14	1
Vulgare × Emmer F_1	35	12–22
Einkorn × Emmer F_1	21	98

with the grain set in fertile species crosses, the degree of sterility may be considered as about 60 to 70 percent in the pentaploid hybrids (see Sax 1921, table 4). The degree of sterility in pentaploid hybrids varies in different species crosses and the above is only a general statement of the facts. In Rosa there is also a high degree of correlation between chromosome duplication and sterility.

In all of the above cases the reduplication of both sets of parental chromosomes does not cause sterility or abnormal chromosome behavior. The triploid hybrid resulting from a cross of a diploid with a tetraploid species or a triploid individual resulting from other causes is characterized by abnormal chromosome behavior and sterility. In wheat, sterility is greater where the proportion of univalent to bivalent chromosomes is 1 : 1 than where the ratio is 1 : 2. In Oenothera, Datura, and in one case in Rosa sterility is associated with the reduplication of a single chromosome. The uniform relation between chromosome behavior and sterility in these cases indicates that similar factors are involved.

In some species hybrids the F_1 is completely sterile even though the parental chromosomes may be very similar. For instance *Crepis tectorum* is thought to have the same chromosome constitution as *C. virens*, but with one reduplicated pair (ROSENBERG 1919), but BABCOCK and COLLINS (1920) have found that a cross between the two species results in an F_1 individual which cannot even complete its vegetative development. Still more remarkable are the recent results of STURTEVANT (1921) with Drosophila. Seven mutant genes of *D. simulans* have been shown to be allelomorphic to mutant genes of *D. melanogaster*, but crosses of these species with a similar chromosome constitution results in sterile F_1 individuals. In other cases species with apparently similar chromosome constitution cannot even be crossed.

Sterility in wheat hybrids

A brief summary of the behavior of the F_2 generation of partially sterile hybrids will be of value in interpreting the correlation between chromosome behavior and sterility. In 1917, 84 seeds from an F_1 plant of Kubanka × Bluestem were planted in the greenhouse at BUSSEY INSTITUTION. Six seeds did not germinate; 18 germinated and grew but did not pass the rosette stage; 61 grew and formed heads, but 14 of these were completely sterile. Of the 47 plants which set grain only one or possibly 2 were as fertile as the parents. The average number of grains per spikelet for the 61 plants that headed was .82 ± .05 as compared with an average of .56 for 30 F_1 plants and 2.45 for the parents. The correlation between sterility and height of the 61 plants that headed was $-.42 \pm .07$, indicating that the more sterile plants were poorly developed vegetatively.

The greater sterility of certain F_2 segregates as compared with the F_1 is shown also in a cross of *T. compactum* × *T. turgidum* grown by GAINES in 1921. The average grains per spikelet for the 293 F_2 plants that headed, was .63 ± .02 as compared with 1.09 ± .05 grains per spikelet for the F_1. In the F_1 no plants were found in the sterility class .0—.2 grains per spikelet, while in the F_2 123 plants were in the lowest sterility class. The conditions under which the F_2 was grown were more favorable than for the F_1. Furthermore a large proportion,—probably more than half,—of the F_1 seed did not grow or did not produce plants which headed.

The relation of chromosome behavior to sterility in wheat hybrids

Let us now gather the facts that bear on the relation of chromosome behavior to sterility and to the origin and the relationships of the various species of wheat. In general, sterility increases as the proportion of univalent chromosomes in the reduction division increases. In both triploid and pentaploid wheat hybrids the 7 univalents apparently pass at random to either pole in one of the reduction divisions. The degree of sterility may vary in pentaploid hybrids involving different parental species (HAYES, PARKER and KURTZWEIL 1920, SAX 1921). Many F_2 individuals may be less fertile than the F_1, but in the F_2 sterility is also associated to some extent with poor vegetative development. With the above cytological and genetic data it should be possible to suggest a cause of sterility in wheat hybrids.

If we assume that the tetraploid and hexaploid species are due simply to a reduplication of the chromosomes of a primary diploid species with only minor changes in the individual chromosomes, then the sterility must be due to quantitative relations between univalents and bivalents rather than any specific constitution of individual chromosomes. That such may be the case is suggested by the chromosome behavior in other genera where the univalents are known to be reduplicated chromosomes of a diploid species. With 7 univalent chromosomes assorting at random only 1 gamete in 64 would contain all or none of the univalents. If only gametes containing all or none of the univalents can develop and function, sterility would be almost complete. Such a relation of the chromosomes would explain practically all of the sterility found in triploid wheat hybrids, but it would not explain the greater fertility found in pentaploid wheat hybrids. Possibly the greater number of bivalents in pentaploid hybrids would permit normal development of gametes with an excess or deficiency of several univalents necessary for a diploid or triploid chromosome combination. If only gametes with 3 or 4 univalents, i. e., gametes with 17 or 18 chromosomes, failed to function, then somewhat more than half of the gametes would be sterile, which in general would agree with the degree of sterility in the F_1. Gametes with larger chromosome numbers might be expected to develop with a greater deviation from the normal gametic number than gametes with a small number of bivalents. In the F_2 individuals the number of univalents would never exceed the ratio found in the F_1 if homologous chromosomes always pair. If then sterility is due to the ratio of univalents to bivalents, the F_2 individuals would never be more sterile than the F_1.

On the other hand, it is probable that more than the mere numerical relations of the chromosomes is involved in the sterility of wheat hybrids. The great differences in morphological and physiological characters indicate that the chromosomes of the various species are unlike. The different wheat groups have existed for long periods of time, apparently without the formation of intermediate types, so that the chromosome constitutions may differ greatly even though the polyploid species have originated by reduplication of the diploid set of a primary species. In the pentaploid F_1 hybrid the 14 chromosomes of the Emmer group presumably pair with the allelomorphs of the Vulgare group which they most nearly resemble. The 7 univalents would then perhaps contain factors primarily characteristic of the Vulgare species. If we assume that the members of the bivalent chromosomes can be interchanged in most cases without causing non-functional chromosome combinations, most of the sterility will be caused by the abnormal behavior of the univalent chromosomes. Gametes with 14 chromosomes, and with perhaps 1 or 2 additional univalents, would be fertile and would carry an excess of Emmer factors, while gametes with 21, 20 or 19 chromosomes would carry largely Vulgare factors. The assumption that gametes with 3 or 4 univalents do not develop is in accord with the cytological finding of KIHARA in F_3 segregates. The fertility increases as the chromosome number progresses from 38 to 42. Some 28-chromosome segregates were highly sterile. This latter case may be due to incompatible relations of the members of the 14 bivalent chromosomes. If therefore chromosome mixtures in the bivalents sometimes disturb gametic development,—although in most cases normal development is correlated with an increase or decrease in number of univalent chromosomes, with greatest fertility in 28- and 42-chromosome individuals,—then most of the data both on character transmission and on sterility can be explained. The union of similar gametes would produce segregates with a predominance of either Emmer or Vulgare characters. Intermediates would be formed by a union of the more extreme types. As a result there would be partial linkage of Emmer characters and of Vulgare characters, although pure parental types would rarely be recovered. A preliminary examination of the genetic data indicates that in the F_2 segregates of an Emmer-Vulgare cross many characters are partially linked. It would be possible only in rare cases therefore to combine the Emmer and Vulgare characters in a fertile homozygous individual. In order to explain why varieties or species with intermediate chromosome numbers such as 34 or 36 are not found, it is necessary to assume further that even the bivalent chromosomes cause sterility unless a complete set is present.

If a complete set of chromosomes is essential not only for gametic but also for somatic development, the greater sterility in F_2 could be explained on the ground that plants without a complete set of diploid or triploid chromosomes vary directly in vegetative development with the completeness of chromosome constitution. In the F_1 two complete sets of chromosomes are present, so that somatic development is normal or is even increased through heterosis. In the F_2 the absence of certain chromosomes may result in various degrees of vegetative development, from plants that do not pass the rosette stage to plants which head out but are poorly developed. *In F_1 sterility is apparently due only to gametic chromosome combinations but in F_2 individuals a weak somatic development would prevent gamete formation although such formation might be possible on a normal plant. Thus the greater sterility in F_2 can be attributed, not to greater gametic sterility, but to a combination of somatic and gametic functions.* The fact that there is a rather high degree of negative correlation between sterility and size of plant indicates that much of the F_2 sterility is due to unfavorable vegetative development of the F_2 individuals.

The assumption that gametes are formed with a predominance of Emmer or of Vulgare factors in the F_1, would explain the absence of homozygous segregates combining the desirable characters of the two different groups. Many hybrids between members of the Vulgare and Emmer group have been made, but few if any of the segregates have contained the desired combinations of parental characters in a homozygous condition.

The above explanation would also agree with the hereditary behavior in F_2.[1] We would expect segregates to form in 3 classes, those with predominance of Emmer characters, those with predominance of Vulgare characters, and those with union of the two types. The latter class of segregates might be considerably reduced by sterility. In the F_2 we actually find considerable linkage of characters. In the F_2 of Amby × Kubanka the type of culm, whether solid or hollow, is partially correlated with length of glume, compactness of spikelet, carination of glume, glume color, pubescence, compactness of head, color of grain and texture of grain. It appears that the 17 characters described can be classed in 3 or 4 partially linked groups. Similar relations are found in other crosses. For instance in a cross of Marquis × Alaska with hollow and pithy straw and hard and soft grain, respectively, there is a high degree of correlation between type of culm and hardness of grain ($r = .48 \pm .04$). It is possible, of course, that the high degree of association of parental characters found in these species crosses is due to chromosome linkage or in some cases to multiple effects of a single factor, but crosses within the Vulgare group have shown only a few linked characters. In either case the association of parental characters indicates the difficulty in combining the desirable characters of the Emmer and Vulgare groups.

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[1] The genetic results here summarized were obtained from an investigation in progress by Dr. GAINES and the writer. Five partially sterile hybrids were grown in the F_2 with a total of 714 individuals. The characters of the F_2 individuals were described and the data were punched on cards and analyzed with the aid of a sorting machine. In two crosses, involving descriptions of 17 characters, each character was correlated with every other character to determine the correlation between the various characters and the correlation of characters with sterility.

The correlation between sterility, as indicated by grains per spikelet, and the number of heterozygous factors in F_2 individuals, should be relatively high if the F_1 gametes are predominantly Emmer or Vulgare in functional composition. Two classes of gametes, consisting largely of factors from either parent, would also be found if EAST's (1915) hypothesis (B) is used to interpret the results. In either case the general method of gamete function is essentially the same, and in the F_2 the segregates resembling the parents should be relatively fertile while those intermediate like the F_1 should be relatively sterile. The correlation between sterility and number of heterozygous factors in an F_2 of *T. vulgare* × *T. turgidum* was found to be practically zero ($r = .03 \pm .06$). Similar results were obtained in other partially sterile wheat hybrids. If most of the 17 characters analyzed are dependent on factors in 2 or 3 chromosomes, little or no correlation would be expected between sterility and number of heterozygous factors.

Although many of the cytological and genetic facts are in accord with the sterility hypothesis presented, more work is necessary to put the chromosome and sterility relationships on a sound basis. Especially is more cytological work necessary in analyzing F_2 individuals. KIHARA (1921) has obtained chromosome counts of 8 F_2 plants in a partially sterile wheat cross and finds the chromosome number ranges from 31 to 42, but not in the frequency that would be expected. KIHARA believes that in the formation of an F_2 plant at least one gamete must have a complete set of either 14 or 21 chromosomes, but if all combinations of univalents and bivalents in the gametes are viable, as KIHARA apparently assumes, and if there is no selective fertilization, then only about 3 percent of the zygotes would develop. Moreover, if the sterility relationships depend on more than mere numerical ratios, and certain specific chromosome combinations cannot function, the degree of fertility will be greatly decreased. If, for instance, all 14 chromosomes of a 14-chromosome gamete must be from the Emmer parent, as KIHARA apparently assumes, then only one gamete in 16,384 would be functional in the F_1

plant. In any case, KIHARA's hypothesis is not in accord with either the cytological facts or with genetic results.

It appears that sterility in wheat hybrids is caused by other factors than those involved in partially sterile Nicotiana hybrids (EAST 1915). In wheat hybrids the abnormal behavior of chromosomes is associated with sterility, although incompatible chromosome combinations probably are factors as in the case of Nicotiana hybrids.

The origin of tetraploidy and hexaploidy in wheat species

The fact that the gametic chromosome number in the species of wheat are in multiples of 7 suggests that the tetraploid and hexaploid species are derived from a primary diploid species by chromosome reduplication. This view is also supported by the fact that several characters are dependent on 2 or 3 independent factors resulting in F_2 ratios of 15 : 1 and 63 : 1. It is also significant that the nearly related cereals have similar chromosome numbers. Rye has 7 haploid chromosomes and in the genus Avena, species are found with 7, 14 and 21 gametic chromosomes (KIHARA 1919). In other genera with polyploid species the tetraploid forms are in most cases known to have originated by chromosome duplication. In the wheat species and in certain other cases there is a striking correlation between chromosome number and cell size, which would indicate that the higher chromosome numbers are the result of duplication and not transverse fragmentation.

On the other hand there is evidence that does not support the view that tetraploidy and hexaploidy in wheat is actually due to chromosomal duplication. In cases where tetraploidy has occurred under experimental conditions the reduplicated sets of chromosomes assort at random in reduction which is not the case in tetraploid wheat species.

If the Emmer and Vulgare groups are the result of polyploidy, then, most if not all characters should be dependent on multiple factors. Of the characters which have been genetically investigated in wheat, 14 are apparently dependent on 1 factor, 4 characters behave as dihybrids, and only 1 character, red grain color, is clearly due to 3 independent factors in some cases. The latter character may also depend on only 1 or 2 factors in certain varieties. In wheat species, where the chromosomes of the polysomic sets assort in pairs, mutations could result in characters dependent on 1 or 2 factors; but in tetraploid species, where the chromosomes of a tetrasomic set assort at random, a single factor could not exist in the homozygous condition. It is possible that chromosomal duplication occurred very early in the history of cultivated wheat and that in the course of time the individual chromosomes came to differ so that they no longer assorted at random within a reduplicated set.

Representations of all of the three groups of wheat can be traced back to prehistoric times (PERCIVAL 1921). Einkorn was probably one of the chief wheats grown in central Europe in the Neolithic period. Emmer was grown in Europe in prehistoric times and was grown in Egypt as early as 5400 B. C. It was the most important cereal in Egypt from the early ages until supplanted by Durum and Vulgare varieties in the Graeco-Roman period. Although representatives of the Vulgare wheat group were known to exist in Europe in prehistoric ages they were not the chief varieties grown until comparatively recent times. The great age of the three wheat groups may also explain why the groups differ so markedly in morphological characters even if they were originally derived from a single primary species by chromosome duplication. Although the species within each group overlap considerably, the differences between the three sterility groups are rather distinct. PERCIVAL has summarized the important characters which differentiate the Vulgare group from the Emmer group as follows: (1) Differences in arrangement of hairs on the leaf; (2) thin-walled, hollow culms; (3) a tough, non-disarticulating rachis; (4) absence of keel on the lower part of the glume in most cases; and (5) the comparatively short awns of the fully bearded varieties and the occurrence of beardless and semi-bearded varieties. The greatest difference is, of course, the high quality of the gluten in most of the Vulgare varieties. No variety of the Emmer group contains gluten of the quality necessary for the production of light spongy bread. The species of the Emmer group are, in general, best adapted to a hot dry climate, and because of susceptibility to cold weather, they are in few cases sown in the fall in the temperate zone.

Many of the characters which distinguish the Vulgare from the Emmer group are found in *T. aegilops ovata* or *T. aegilops cylindrica* and accordingly PERCIVAL has concluded that the Vulgare group is a hybrid race resulting from an early cross or crosses of members of the Emmer group and *T. aegilops*. The hybrids of the present species of the Emmer group or even *T. dicoccoides* with *T. aegilops* are, however, nearly or quite sterile in the F_1.

Chromosome number and adaptability in wheat species

The characters of the Vulgare group are subject to very wide variation and the great number of varieties and intermediate forms are believed by

Percival to be due to their hybrid origin. The great variability and adaptability of the Vulgare wheats may, however, be due to other causes. East (1915) suggested that variability of a species would be correlated with the chromosome number and Dorsey in 1916 suggested that the adaptability of a species might be correlated with chromosome number. With an increase of chromosome number the number of factorial combinations would increase, thus resulting in a greater range of adaptation. There is certainly a high degree of correlation between chromosome number and adaptability in the species of wheat. The number and distribution of the varieties of the cultivated species of wheat are shown in table 7.

Table 7

Number and distribution of varieties of the Einkorn, Emmer and Vulgare groups. (*Data from* Percival 1921.)

	T. monococcum	*T. dicoccum*	*T. durum*	*T. polonicum*	*T. turgidum*	*T. pyramidale*	*T. vulgare*	*T. compactum*	*T. sphaerococcum*	*T. Spelta*
Number of "varieties"	3	40	22	23	27	5	26	20	6	12
Groups		Emmer					Vulgare			
Haploid chromosome number	7	14					21			
Distribution of "forms"										
S. E. Europe, S. W. Asia	*	10					9			
Southern Europe	*	77					42			
Russia		7					12			
Central and N. Europe		27					102			
England		13					91			
India		2					4			
Australia and New Zealand		3					63			
South Africa		4					23			
Japan and China							11			
North America		7					109			
South America		1					7			
Total		151					536			

*Species of Einkorn grown but no cultivated "forms" described.

Percival (1921) recognizes only two wheat species, *T. aegilopoides* and *T. dicoccoides*, and suggests that the other types might be classed as "cultivated species." Under the term "forms" PERCIVAL classes what are commonly known as varieties, such as Marquis, Bluestem, Kubanka, etc. It will be seen in table 7 that the number of the varieties is greatest in the Emmer group, but that the Vulgare group contains almost four times as many "forms." The distribution of the different groups is also significant. The members of the Einkorn and Emmer groups are found in greatest numbers in the regions where wheat originated. In general the region has a warm dry climate. The members of the Emmer group are comparatively rare in Australia, England, South Africa, Japan and North America. Members of the Vulgare group are found all over the world wherever wheat is grown and under diverse climatic conditions. Although the Vulgare group is more variable and adapted to a greater range of conditions they are not always the highest yielders. Certain varieties of *T. turgidum* are the most productive of all wheats, and under certain conditions varieties of *T. durum* will outyield the Vulgare varieties. The correlation between chromosome number and adaptability in wheat species may not depend on a causal relationship. The Vulgare group may be more highly selected and more widely distributed because it is the only wheat suitable for making light bread. The greater variation and adaptability would also be expected if the Vulgare group is a vast hybrid race as PERCIVAL has suggested. A study of chromosome number in relation to variations and adaptability in polyploid species under natural conditions would be of interest.

The value of tetraploidy

Since tetraploidy usually results in increased size of plant tissues and possibly permits greater adaptability, the development of tetraploid species may be of value. WINKLER (1916) has obtained tetraploid forms of tomatoes and nightshade in connection with his work on graft hybrids. The tetraploid forms were larger than diploid forms but unfortunately they were sterile. A number of investigators have been able to induce changes in the chromosome number of somatic cells by treatment with various reagents (SAKAMURA 1920) but it appears to be questionable if such changes can be maintained and established in later generations. CASTLE (1921) has suggested that the French prune mutation described by SHAMEL (1919) is the result of tetraploidy. It is not improbable that the best cases of bud mutations involving increased productivity (if such mutations actually occur) will be found to be associated with tetraploidy or chromosome duplication.

DESCRIPTION OF PLATES

Preparations from pollen mother cells at the time of the reduction divisions. The drawings were made with the aid of a camera lucida. All figures were drawn from single sections. Magnification 750 diameters. No reduction. The reproduction of these drawings has been made possible by financial assistance from BUSSEY INSTITUTION.

PLATE 1

Triticum monococcum

FIGURE 1.—The 7 paired chromosomes at diakinesis.

FIGURE 2.—Metaphase, polar view. Heterotypic division.

FIGURE 3.—Metaphase, side view.

FIGURE 4.—Late anaphase, 7 chromosomes at each pole.

FIGURE 5.—Polar view of late anaphase showing the 7 chromosomes split for the homoeotypic division.

FIGURE 6.—Telophase of the homoeotypic division.

The Emmer group

FIGURE 7.—Diakinesis. 14 pairs of chromosomes. *T. durum.*

FIGURE 8.—Metaphase, polar view. 14 chromosomes.

T. polonicum

FIGURE 9.—Metaphase, polar view. *T. durum.*

FIGURE 10.—Metaphase, side view. *T. polonicum.*

FIGURE 11.—Anaphase of heterotypic division. *T. polonicum.*

FIGURE 12.—Interkinesis. Resting nuclei. *T. polonicum.*

FIGURE 13.—Telophase of homoeotypic division. *T. durum.*

The Vulgare group

FIGURE 14.—Metaphase, polar view. 21 chromosomes. *T. Spelta.*

FIGURE 15.—Metaphase, polar view. 21 chromosomes. *T. vulgare.* (Preston)

FIGURE 16.—Metaphase, side view. *T. vulgare.* (Preston)

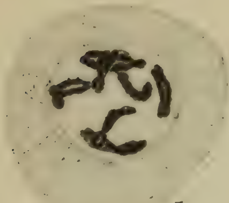
FIGURE 17.—Late anaphase of heterotypic division, side view. *T. vulgare.* (Preston)

FIGURE 18.—Late anaphase, polar view. *T. vulgare.* (Preston)

FIGURE 19.—Telophase. *T. vulgare.* (Marquis)

FIGURE 20.—Metaphase. Homoeotypic division, polar view. *T. vulgare.* (Amby)

FIGURE 21.—Telophase, homoeotypic division. *T. vulgare.* (Amby)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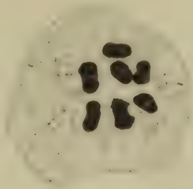
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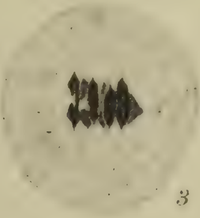
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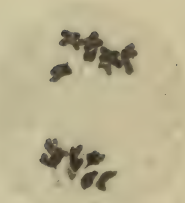
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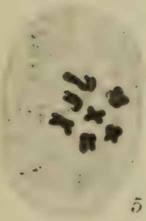
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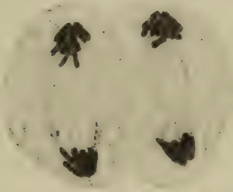
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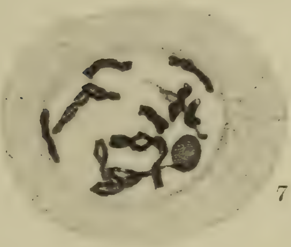
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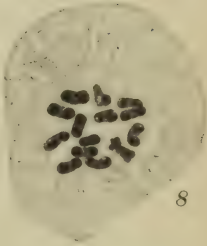
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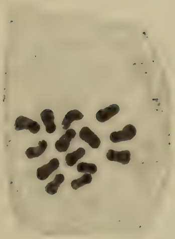
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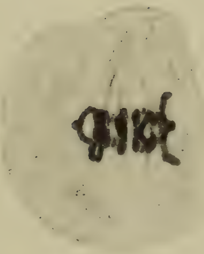
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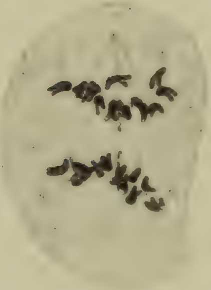
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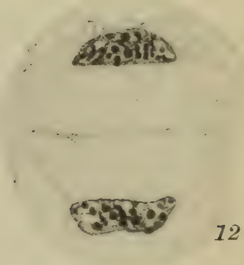
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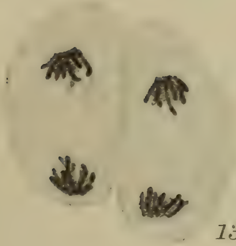
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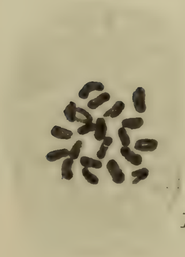
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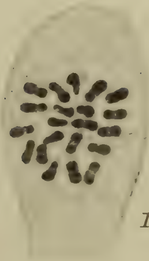
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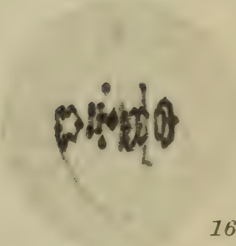
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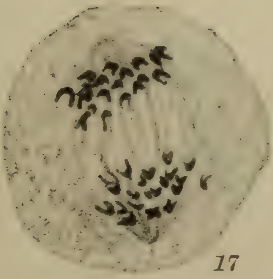
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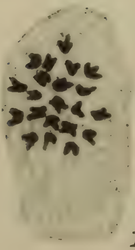
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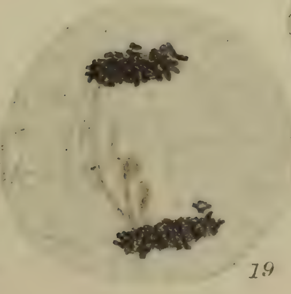
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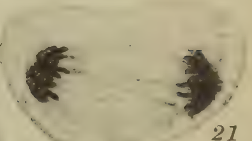
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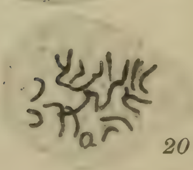
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The value of polyploidy may have certain limitations. As TUPPER and BARTLETT (1918) have suggested, a few doublings in chromosome number would increase the size of the cell so that respiration and nutrition would be retarded. In Crepis and Chrysanthemum, however, the chromosome number has apparently been duplicated many times without disastrous results. MULLER (1918) believes that tetraploidy would hinder evolution because recessive mutants could rarely manifest themselves. If recessive factors have some effect in the presence of dominant factors, which is frequently or perhaps usually the case, then increased variability would result even if the pure recessive could rarely be isolated.

There has been considerable discussion as to the origin of tetraploid species especially in Oenothera. GATES (1915) argues that *Oe. gigas* must have originated by a doubling of chromosomes at an early stage in somatic development of a diploid species, because of the rare occurrence of diploid gametes. This interpretation is undoubtedly the most reasonable in cases where tetraploid individuals have suddenly originated from a diploid species. If a diploid gamete united with a normal haploid gamete and the chromosomes in the trisomic sets assorted at random 25 percent of the F_2 segregates would be tetraploid. The occasional occurrence of diploid gametes has been observed in several diploid species and would afford an opportunity for the formation of tetraploid species although the latter would originate from a triploid individual and not directly from the original diploid species.

Pollen-grain variability

The pollen of F_1 plants of species hybrids is much more variable than the pollen of pure species.

The remarkable relation between heterosis or degree of germinal mixture and variability of pollen grains is especially interesting because the pollen grains belong to the gametophytic generation. In most cases recorded the characters of the pollen grain are determined by the mother plant. BATESON (1909) has found that the shape and color of pollen in the sweet pea behave as sporophytic characters. EAST (1916) has found a similar behavior of pollen in Nicotiana and also finds that self-sterility behaves as a sporophytic character (EAST 1919). On the other hand BELLING (1914) has found segregation of normal and aborted pollen grains in semi-sterile F_1 hybrids, and PARNELL (1921) finds segregation of equal numbers of starchy and glutinous pollen grains in an F_1 rice hybrid. The occurrence of the starchy and glutinous pollen grains

DESCRIPTION OF PLATE 2

T. monococcum (7-chromosome gametes) × *T. turgidum* (14-chromosome gametes)

FIGURE 22.—Metaphase of heterotypic division with 7 bivalents (1 divided) and 7 univalents at the poles.

FIGURE 23.—Late anaphase with approximately 10 chromosomes at either pole due to the division of the bivalents and the random assortment of the univalents without dividing.

FIGURE 24.—Early telophase. Split chromosomes, originally members of the bivalent chromosomes, just reaching the poles.

FIGURE 25.—Telophase. No lagging chromosomes.

FIGURE 26.—Anaphase of homoeotypic division. Vulgare group (21-chromosome gametes) × Emmer group (14-chromosome gametes), F_1 plants.

FIGURE 27.—Metaphase, polar view, of homoeotypic division with about 10 chromosomes in each figure.

FIGURE 28.—Metaphase of heterotypic division, polar view. Fourteen bivalents and 7 univalents. Amby × Kubanka.

FIGURE 29.—Metaphase showing bivalents and univalents. Bluestem × Alaska.

FIGURE 30.—Metaphase of heterotypic division showing irregularly distributed univalents. Note the attachment of the spindle fibers and apparent tension of the chromosomes. Amby × Kubanka.

FIGURE 31.—The bivalents have divided leaving the 7 univalents and apparently several additional unpaired chromosomes on the equatorial plate. Amby × Kubanka.

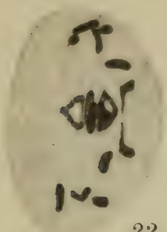
FIGURE 32.—The 14 bivalents have divided and reached the poles while the 7 univalents are dividing equationally. Amby × Kubanka.

FIGURE 33.—A similar stage in Bluestem × Kubanka.

FIGURE 34.—A similar stage in Bluestem × Kubanka.

FIGURE 35.—The 7 univalents have divided and have nearly reached the poles. Bluestem × Alaska.

FIGURE 36.—Homoeotypic division showing univalents which do not divide but pass at random to either pole and join the original 14 bivalents.



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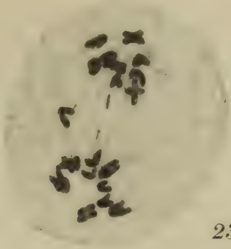

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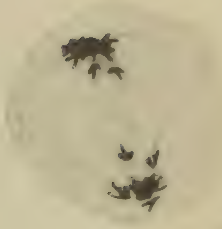

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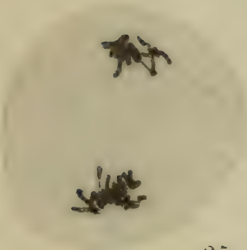

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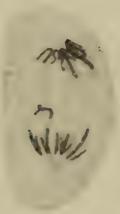

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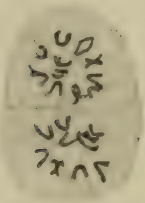

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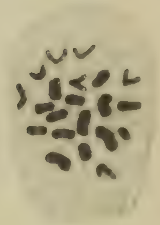

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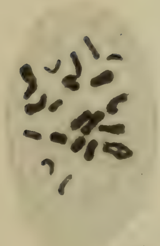

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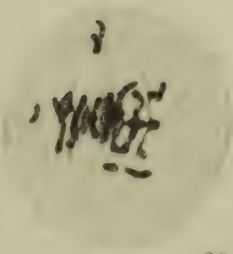

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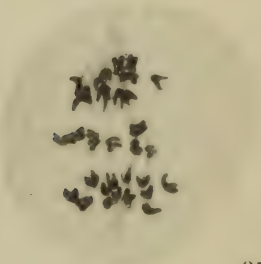

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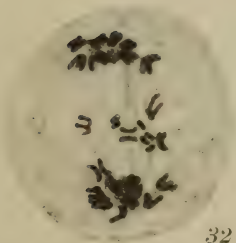

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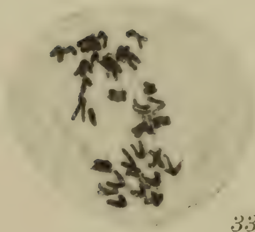

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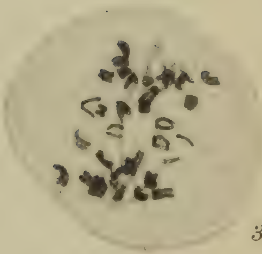

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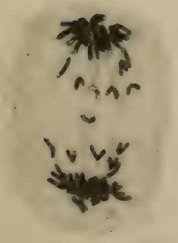

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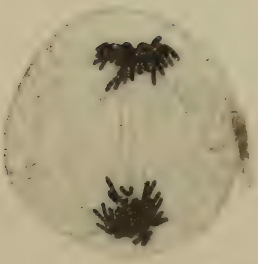

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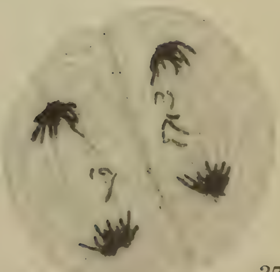


37

H. J. S and K. S.

is undoubtedly due to the effect of the segregated factors. In the wheat hybrids the factors for growth apparently segregate into various proportions of maternal and paternal factors in the F_1 and the various combinations of these factors may cause increasd variation in size of pollen grains, ranging from completely aborted grains to those much larger than normal. The increased variability is due here to combinations of non-homologous chromosomes acting in the gametophytic generation and to differences in chromosome number. In cases where the gametophytic characters are controlled by the sporophyte the characters may be those primarily caused by the sporophyte, such as pollen-grain shape and possibly color. In other cases certain characters, such as growth and starch-formation, may be controlled by the gametophytic chromosomes at an early stage in the development of the gametophyte, while perhaps other characters cannot be brought into expression until the necessary development of the organism has been attained.

SUMMARY

The chromosome number and behavior has been determined for the cultural species of Triticum and for certain partially sterile hybrids.

The gametic chromosome number is 7 for *T. monococcum*; 14 for the Emmer group, consisting of *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum;* and 21 for the Vulgare group, consisting of *T. vulgare*, *T. compactum* and *T. Spelta*. Rye (*Secale cereale*) has 7 gametic chromosomes.

In the F_1 hybrid of *T. monococcum* × *T. turgidum* 7 chromosomes are contributed by one parent and 14 by the other parent. In the heterotypic division of the pollen mother cells there are 7 bivalents and 7 single chromosomes. The 7 bivalents divide normally, but the 7 univalents pass at random to either pole without dividing. In the homoeotypic division there are usually about 10 or 11 chromosomes which apparently divide normally in most cases. Tetrads and one-nucleate pollen grains appear to be normal, but very few normal mature pollen grains are formed.

In F_1 hybrids between members of the Emmer group and members of the Vulgare group, 14 chromosomes are contributed by one parent and 21 by the other parent. In the first meiotic division there are 14 bivalent and 7 univalent chromosomes. The bivalents divide normally, but the univalents do not become oriented on the equatorial plate until the bivalents have divided. The 7 lagging univalents divide equationally and 7 chromosomes pass to each pole. In the homoeotypic division the

DESCRIPTION OF PLATE 3

Micro-photographs of chromosomes in the reduction divisions of the pollen mother cells in wheat species, rye, and wheat hybrids. Photographs not retouched.

Figure 37.—Late anaphase showing the 7 chromosomes in Einkorn.

Figure 38.—Late anaphase, side view. Einkorn.

Figure 39.—Late anaphase with 14 chromosomes at one pole. Polish.

Figure 40.—Metaphase, polar view. 21 chromosomes. Preston.

Figure 41 A, B.—Diakinesis in rye, showing the 7 pairs of twisted chromosomes.

Figure 42.—Metaphase showing univalents and bivalents in (Amby × Kubanka) F_1.

Figure 43.—Bivalent chromosomes have divided and reached the poles while the split univalents are on the heterotypic plate. (Amby × Kubanka) F_1.

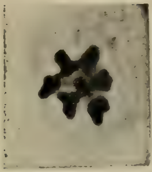
Figure 44.—Metaphase, anaphase, and telophase in 3 adjacent pollen mother cells in an anther of Preston.

Figure 45.—Same as figure 43.

Figure 46.—Telophase, no lagging chromosomes. (Amby × Kubanka) F_1.

Figure 47.—Metaphase, side view of the homoeotypic division showing several irregularly distributed chromosomes. (Amby × Kubanka) F_1.

Figure 48.—Homoeotypic division showing lagging chromosomes. Same as figure 36. Amby × Kubanka) F_1.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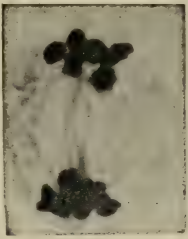
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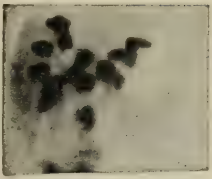
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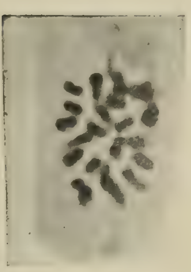
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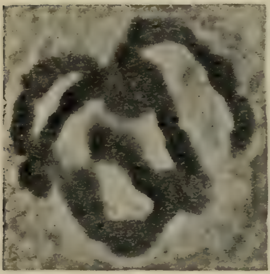
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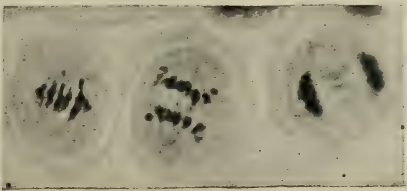
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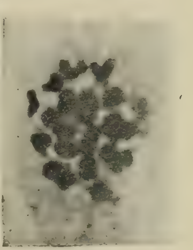
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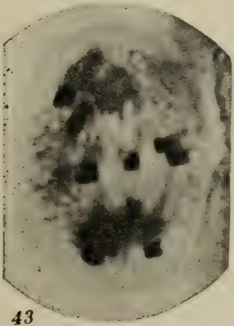
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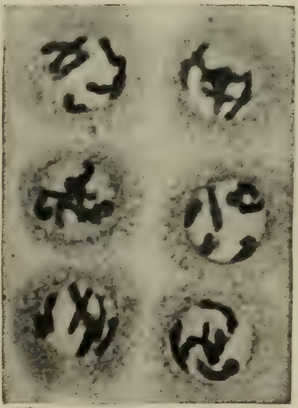
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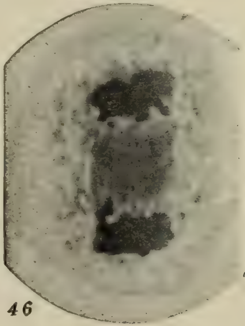
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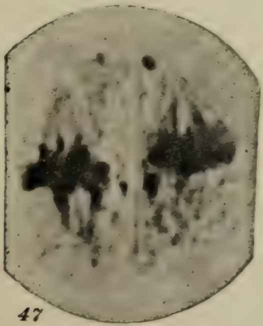
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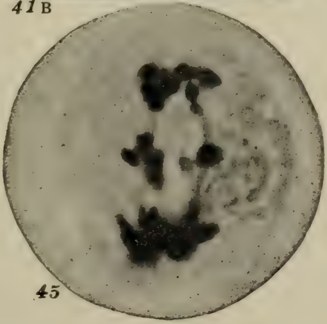
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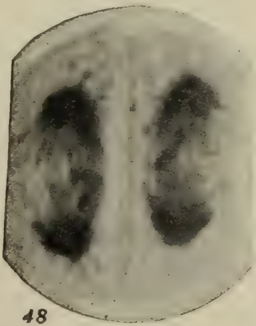
48

J. W. B. and K. S. photo.

original members of the bivalents divide normally, but the remaining 7 chromosomes pass at random to either pole without dividing. As a result the chromosome number of the microspores varies from 14 to 21. The tetrads and one-nucleate pollen grains appear to be normal, but in later stages about 20 percent of the pollen grains are obviously imperfect and undoubtedly a larger percentage is non-functional.

The size of the pollen grains is closely correlated with the chromosome number in the various species of wheat.

The pollen grains of fertile species hybrids are more variable than the pollen grains of the parental species due to various degrees of compatibility of the combinations of non-homologous chromosomes in the gametophytic generation. In partially sterile hybrids where the parental species differ in chromosome number, the pollen grains are extremely variable, due to difference in chromosome number and to more or less compatible chromosome combinations.

The sterility in the hybrids described can be accounted for on a hypothesis involving, (1) the numerical or unbalanced relations of the chromosomes resulting from the irregular meiotic divisions, and (2) the specific interrelations of the parental chromosomes. In the numerical relations the development of gametes varies as the chromosome number approaches the normal gametic number (7, 14 or 21). It is assumed that the greater the gametic chromosome number the greater can be the deviation from the normal number. The specific relations of the gametic chromosomes will depend on the extent that chromosomes from one parent can be substituted for those of the other parent. Gametic perfection will vary as the chromosome constitution approaches that of the parental forms. The 7 univalents in the Emmer-Vulgare F_1 hybrids presumably carry most of the factors which differentiate the Vulgare characters from the Emmer characters.

This hypothesis will explain (1) the differences in sterility of the various species hybrids, (2) the partial association of the original parental characters in the F_2 segregates, (3) the absence of varieties or species with intermediate chromosome numbers, and (4) the difficulty in obtaining homozygous segregates combining the desirable characters of the parental species in partially sterile wheat hybrids.

In all cases the F_1 plants are unusually vigorous and sterility is not due to poor vegetative development, but is caused by the formation of non-functional gametes. Sterility in F_2 segregates may be greater than in F_1 individuals due, not to greater gametic sterility *per se*, but to a combination of weak somatic development and gametic sterility.

The evidence for and against the occurrence of chromosome duplication in wheat species is presented and discussed.

There is a high degree of correlation between chromosome number and adaptability in the wheat species, but the relation may not be a causal one.

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STERILITY IN WHEAT HYBRIDS

III. ENDOSPERM DEVELOPMENT AND F_2 STERILITY

A THESIS SUBMITTED BY

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TO THE FACULTY OF THE BUSSEY INSTITUTION OF HARVARD UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF SCIENCE
1922

Reprinted from GENETICS **7**: 553-558, November, 1922

STERILITY IN WHEAT HYBRIDS. III. ENDOSPERM DEVELOPMENT AND F_2 STERILITY

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Received April 24, 1922

Crosses of wheat species with different chromosome numbers result in small or wrinkled seeds. These seeds consist of the F_1 endosperm and the embryo of the F_1 plant. Although the F_1 endosperm is usually poorly developed, the F_1 plant is even more vigorous than the parents. The F_1 plants are partially sterile and bear grains (F_2 endosperms) which vary greatly in size. Since small wrinkled grains are characteristic of partially sterile hybrids the small wrinkled grains borne on the F_1 plant might be expected to produce partially sterile F_2 plants, while the large plump grains would produce relatively fertile F_2 individuals.

It is well known that wide species crosses in wheat result in partially sterile F_1 plants and that in F_2 all degrees of sterility are found, ranging from plants which never pass the rosette stage, to those quite as fertile as the parents. A study of sterility in view of the chromosome relationships should be of value.

The parental varieties of wheat used were *Triticum durum* Desf. (Kubanka) and *T. vulgare* Vill. (Pacific Coast Bluestem). The crosses were made in 1916 and the F_1 was grown at BUSSEY INSTITUTION in 1917. The F_2 was grown in the greenhouse in 1917–1918.

WEIGHT OF SEEDS OF PARENTS AND HYBRIDS AND F_1 STERILITY

The degree of sterility, as indicated by the grains set per spikelet, and weight of grains of parents and the F_1 hybrids are shown in table 1. The F_1 plants are about one-fourth as fertile as the parents, although they are more vigorous vegetatively as indicated by the greater number of heads per plant. There is little difference in the fertility of the reciprocal hybrids. For the 30 F_1 plants the average number of grains per spikelet was found to be $0.57 \pm .03$ with a standard deviation of $0.25 \pm .02$. The sterility of the F_1 plants varied from .07 to 1.00 grain per spikelet. It appears probable that with larger numbers some F_1 individuals would

TABLE 1

Weight of grain and sterility in parents and hybrids.

NO. OF PLANTS	NO. OF HEADS	NO. OF SPIKELETS	NO. OF GRAINS	GRAINS PER SPIKELET	WEIGHT OF GRAINS IN 5-MG UNITS															NUMBER	MEAN	STANDARD DEVIATION
					2.5-7.5-	7.5-	12.5-	17.5-	22.5-	27.5-	32.5-	37.5-	42.5-	47.5-	52.5-	57.5-	62.5-	67.5-	72.5-77.5-			
					Kubanka																	
6	9	144	386	2.68					1	8	25	30	45	55	89	82	20	1		356	48.9±.3	8.9±.2
					Bluestem																	
7	23	354	789	2.23				1	2	14	27	51	115	123	100	24	2			458	45.2±.2	7.4±.2
					(Kubanka × Bluestem)F_1 endosperm																	
							2	4	7	6	4	1								24	24.4±.9	6.4±.6
					(Kubanka × Bluestem)F_1 (F_2 endosperm)																	
15	152	2323	1413	.61	4	19	31	50	106	124	138	154	183	161	175	121	103	33	11	1413	41.4±.3	14.5±.2
					(Bluestem × Kubanka)F_1 (F_2 endosperm)																	
15	131	1932	990	.51	9	22	34	50	77	109	115	123	114	110	95	70	37	19	6	990	38.1±.3	14.7±.2

be totally sterile, although the percentage of such plants would be small. The variation in sterility of the F_1 plants is apparently not due to differences in vegetative development, because the correlation between sterility, as indicated by the number of grains set per spikelet, and vegetative vigor, as indicated by the number of heads per plant, is practically zero ($r = -.08 \pm .12$).

Kubanka has somewhat larger seeds than Bluestem, but the variability of grain weight does not differ greatly although the difference is statistically significant. The grains resulting from the immediate cross (F_1 endosperm) are but little more than half as heavy as the grains of the parents and are no more variable. The grains borne by the F_1 plants[1] (F_2 endosperm) are extremely variable, with a standard deviation of nearly twice that of the parental grain weight. The average grain weight of the F_1 plants is greater when Kubanka is used as the female parent, but the difference, although statistically significant, may depend on environmental and not genetic factors.

STERILITY IN THE F_2 GENERATION

The seed from an F_1 plant of Kubanka × Bluestem was planted in the greenhouse under very uniform and favorable conditions. The F_1 plant selected was representative of all F_1 plants, both in vegetative development and sterility. Of the 84 seeds planted 6 failed to germinate, 78 seeds germinated but of these only 61 produced heads and only 47 produced grain. In the entire F_2 only 1 or perhaps 2 plants were as fertile as the parents. The descriptions of the F_1 grain (F_2 endosperm) and the resulting F_2 plants are given in table 2.

The sterility in F_2 ranged from plants which were totally sterile to one which set 2.66 grains per spikelet. The mean sterility of the 61 plants that headed was found to be .83 with a standard deviation of .64. Thus in the F_2 a larger proportion of plants is totally sterile than in the F_1. Moreover, many F_2 plants fail to develop normally and a large percentage fails to produce heads. Under field conditions probably half of the F_1 grain fails to produce viable F_2 plants, and under unfavorable climatic conditions nearly all F_1 grains fail to develop, even though standard varieties may show more than 50 percent germination under the same conditions. Much of the extreme sterility in F_2 is undoubtedly due to

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[1] The mean weight and variability of seeds of parents and F_1 plants were relatively uniform, so all individuals of each class were grouped together. The variability in seed weight is not due to differences in average seed weight of individual plants.

TABLE 2

Kubanka × *Bluestem*—F_1 and F_2.
(*Greenhouse* 1917.)

F_1			F_2				F_1			F_2			
Grain No.	Weight in mg*	Wrinkled or plump	Height of plant in cm	No. of spikelets	No. of grains	Grains per spikelet	Grain No.	Weight in mg	Wrinkled or plump	Height of plant in cm	No. of spikelets	No. of grains	Grains per spikelet
1	15	W	127	120	156	1.30	43	45	P	96	54	62	1.15
2	20	W					44	45	P	128	80	20	.25
3	20	W					45	45	P	128	60	45	.75
4	20	P					46	45	P	117	+	0	.00
5	25	W	132	106	142	1.34	47	45	P	*9*			
6	25	W	131	90	111	1.23	48	45	P	137	148	213	1.44
7	25	W	118	72	78	1.10	49	50	W	145	66	21	.32
8	30	W	123	68	23	.34	50	50	W	109	+	0	.00
9	30	W					51	50	W	128	+	0	.00
10	30	W	124	100	200	2.00	52	50	W	54	+	0	.00
11	30	P	136	115	58	.50	53	50	W	140	100	41	.41
12	30	P					54	50	W	*14*			
13	35	W	119	92	127	1.38	55	50	W	*13*			
14	35	W	115	124	177	1.42	56	50	P	107	88	16	.18
15	35	W	130	74	6	.08	57	50	P	80			
16	35	W	128	47	125	2.66	58	50	P	30			
17	35	W	130	150	68	.45	59	50	P	115	+	0	.00
18	35	P	105	74	10	.14	60	50	P	40			
19	40	W	126	116	152	1.31	61	50	P	129	188	391	1.81
20	40	W	136	102	96	.94	62	50	P	*13*			
21	40	W	*10*				63	55	W	*14*			
22	40	W	122	122	185	1.52	64	55	W	115	151	253	1.70
23	40	W	35				65	55	W	142	102	37	.37
24	40	W	*10*				66	55	W	114	86	118	1.37
25	40	W	110	60	77	1.28	67	55	W	*14*			
26	40	W	105	80	57	.71	68	55	P	124	138	180	1.30
27	40	W	40				69	55	P	105	100	131	1.31
28	40	W	*11*				70	55	P	87	+	0	.00
29	40	W					71	55	P	147	36	52	1.44
30	40	W	127	130	183	1.41	72	55	P	145	192	306	1.60
31	40	P	106	112	73	.65	73	55	P	50	+	0	.00
32	40	P	*7*				74	60	W	130	94	111	1.18
33	40	P	100	+	0	.00	75	60	W	115	+	0	.00
34	40	P	117	80	15	.19	76	60	P	60			
35	40	P	60	+	0	.00	77	60	P	135	126	216	1.71
36	40	P	40				78	60	P	86	+	0	.00
37	45	W	132	70	50	.70	79	60	P	153	104	136	1.31
38	45	W	121	68	78	1.14	80	65	W	90	+	0	.00
39	45	W	100	130	59	.45	81	65	W	115	100	167	1.67
40	45	W	137	126	67	.53	82	65	W	155	34	10	.30
41	45	W	108	100	29	.29	83	65	P	61	+	0	.00
42	45	P	130	84	138	1.64	84	70	W	63	+	0	.00

poor vegetative development of certain plants. Such a relation is indicated by the relatively high correlation between sterility and height of the 61 F_2 plants which headed ($r = .42 \pm .07$). Among the 47 plants which set grain the high sterility of certain individuals does not seem to be due to poor vegetative development, since there is no significant correlation between height and sterility of the 47 F_2 plants ($r = .04 \pm .10$).

The 84 F_1 grains were extremely variable in weight and were also variable in shape,—whether wrinkled or plump. In table 2 the F_1 grains are arranged in order of weight and arbitrarily classified as wrinkled or plump. The average weight of the wrinkled grains was found to be but little less than the weight of the plump grains. The correlation between weight and plumpness of grain was found to be rather low ($r = .23$). The correlation was determined by a method suggested by PEARSON (1909).

Among the F_1 seeds which failed to germinate, a majority are in the lower weight classes, but among the 78 plants which grew there is little or no correlation between weight of F_1 grains and height of the resulting F_2 plant ($r = -.10 \pm .05$). The correlation is not greatly increased by using only the 61 F_2 plants which headed ($r = -.20 \pm .08$.) The size of the F_1 grain gives little indication of the probable size of the F_2 plant. The plumpness of F_1 grains has little or no effect on the size of F_2 plants. For the 78 F_2 plants the correlation between wrinkled or plump F_1 grain and height of F_2 plants was found to be only .10, and for the 61 plants which headed, $r = .10$.

A similar correlation was found between F_1 grain weight and F_2 sterility. For the 47 F_2 plants that headed, the correlation between F_1 grain weight and F_2 sterility was $.03 \pm .10$, and for all 78 plants, $r = -.19 \pm .08$. The correlation between wrinkled or plump F_1 grains and F_2 sterility was found to be .24 for the 61 F_2 plants which headed. Although small wrinkled grains are characteristic of partially sterile hybrids there is little or no correlation between F_1 grain weight or shape and the height or sterility of the resulting F_2 plants.

DISCUSSION AND SUMMARY

In a cross of Kubanka × Bluestem a male gamete with 21 chromosomes unites with an egg cell containing 14 chromosomes. In the endosperm there are 28 chromosomes of maternal origin and 21 chromosomes of paternal origin while in the reciprocal cross 42 chromosomes are contributed by the polar nuclei and only 14 by the sperm nucleus. Such unbalanced conditions of the chromosome sets might be expected to result in abnormal endosperm development, although in pure species the triploid condition of the endosperm does not result in abnormal development, unless endosperm formation itself is considered abnormal. Endosperm formation is not due simply to the triploid condition, however, because triploidy is found in the vegetative cycle of a number of plants.

The greatly increased variability of the F_1 grain (F_2 endosperm) can be attributed to differences in chromosome number, to recombinations of factors for growth, and to more or less compatible chromosome combinations. Although the maximum mixture of parental chromosomes is found in the F_1 endosperm the F_2 endosperms may in many cases be significantly smaller than F_1 endosperms, due to inharmonious chromosome relations.

Since poorly developed F_1 endosperm is associated with sterility and unusual vegetative vigor of the F_1 plant, a high degree of correlation would be expected between F_2 endosperm development and sterility and vegetative development of F_2 plants. An analysis of the data shows, however, that there is little or no correlation between F_2 endosperm development and F_2 sterility or vegetative development. Apparently the results of the peculiar chromosome relationships in endosperm formation do not indicate the nature of the chromosome relations of the accompanying embryo. This relation may be due to the greater opportunity for an unbalanced condition of maternal and paternal chromosomes in the endosperm.

The increased sterility in F_2 individuals as compared with the F_1, and especially the weak somatic development of many plants, may be attributed to chromosome combinations incompatible for vegetative development. The chromosome compatibility may depend on numerical or balanced relation of the chromosomes or on specific chromosome relationships. In F_1 plants two complete sets of chromosomes are present and the F_1 plant is unusually vigorous even though it is partially sterile. In F_2 individuals a complete set of chromosomes of either parent would rarely be found and the perfection of somatic development would be expected to vary with the completeness of the parental sets of chromosomes. Thus in F_2 many individuals are completely sterile because of weak vegetative development as well as gametic sterility.

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MENDEL AND HIS CONTEMPORARIES

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Reprinted from THE SCIENTIFIC MONTHLY, pages 225-236, Vol. XVI, No. 3,
March, 1923.

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Reprinted from THE SCIENTIFIC MONTHLY, pages 225-236, Vol. XVI, No. 3, March, 1923.

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By Professor E. M. EAST

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IN law the *death duty* is a tax imposed on the transfer of property at the owner's death. It is a tribute which the legatee pays to the public in return for an acknowledgment of title to his inheritance. To-day we publicly pay a death duty for the intellectural legacy which we as biologists have received from Mendel and his contemporary fellow-workers. We meet to proclaim our indebtedness to the men who found the study of heredity buried in the depths of a hazy mysticism, and left it one of the most firmly established branches of quantitative biology. There is an element of affection apart from the sense of justice in making this acknowledgment. We know that nothing that we can say here will add to or detract from the merits of these men of the latter part of the nineteenth century, but we wish to say a few words of homage as a free-will offering to the excellence of past greatness.

I was quite proud of the above paragraph when it was first written. Having listened attentively to several political orators on Memorial Sunday, I felt that it had just the proper shade of artistic solemnity expected on such occasions. In fact, it seemed as if both custom and decency demanded a series of eulogies attuned to a motive resembling the Dead March from ''Saul.'' But I am told that this apparently decorous procedure is a mere subterfuge which deceives no one who has been taught the rudiments of interpreting the subconscious. What we really do in a case like this, I learn, is to use it as an opportunity for releasing various overcompensations from the springs of our own vanity. These supposed tributes are defense reactions. Their true purpose is to show how much better is the scholarship of the present day—that is to say, our own scholarship—than any which went before. Our motto is *De mortuis nil nisi bonum,* but our memorial wreaths are twined with poison ivy. We are not far from following the intent of the epitaph of the gentleman of the frontier, without a single redeeming quality, without a single friend, whose fellow-townsmen marked his last resting place with the truthful inscription: ''In memory

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[1] Papers in honor of the centenaries of the birth of Gregor Mendel and of Francis Galton, presented at a meeting of the American Society of Naturalists held at Boston, on December 29, 1922.

of John Black the citizens of Crimson Gulch are happy to erect this monument.''

One is doomed, therefore; if he escapes Scylla, he is certain to be wrecked on Charybdis. According to my informant, if one praises, his encomiums are accounted like those of him who expatiates on the superb tennis of the opponent he has just beaten; if he is critical, he lacks the good taste to conceal either his envy or his egotism. It is disturbing thus to be initiated into the secret sordidness of the human soul. I had intended to utilize this auspicious occasion to inquire into the *reasons* for Gregor Mendel's successful masonry in laying a solid foundation for twentieth-century genetics. It appeared to be a worthy academic question which might have a modicum of importance in determining future trends in biology. I could have entered the affair stimulated by the valor of ignorance, like the Honorable William Jennings Bryan, and have said whatever seemed fitting; now I suppose I ought to be as circumspect as a speaker at a birth-control meeting with Anthony Comstock on the front seat.

Fortunately one need neither admit nor deny the contentions of these psycho-analysts. Let the actual personal motives for our actions be what they may, there is an obvious reason both for praising and for criticizing in this particular memorial celebration, which ought to be satisfactory to all concerned. Whether we belong to the aristocracy or the proletariat of science, whether our day be the nineteenth or the twentieth century, let us do one another the honor of believing that each can be counted among those who seek the plain unqualified truth. If, therefore, we do not judge those of the past as carefully as those of the present, we do both an injustice; in plain language, we are insincere ourselves, and we assume that our predecessors were such false scientists as to prefer hollow eulogies which lead nowhere to critical discussions which might aid in banishing error and fallacy.

When one speaks of co-mendelian genetic biology, he must include the sixty years previous to the beginning of the twentieth century if he is to obtain any enlightenment on the subject of Mendel's triumph. During this period there were three types of work in progress which contributed directly to the establishment of genetics—experimental breeding, morphology and demographic mathematics. If one should undertake to enumerate the investigations which contributed their mites indirectly, he would be compelled to list every advance in knowledge made, for science seems to be like the colonial protozoan, faring best when cultivating interdependence.

The experimental breeding of the time was plant breeding. Animal breeding, of course, was much older; but as a method by which to discover new facts in pure science, it remained in the same stage of moribund quiescence from the time of the Babylonians, until rejuvenated by the quantities of interstitial tissue inserted by the zoologists of the present day. Plant breeding in England, France and Germany, on the other hand, had established several very interesting truths, such as the similarity of reciprocal crosses, the high variability of hybrids of the second generation when compared with that of the first, the dominance of characters and the reappearance of characters after being lost in the melting pot of the first hybrid generation. The names of the workers who made these discoveries are familiar. Among them are Gärtner, Godron, Lecoq, Herbert, Naudin, Vilmorin, Klotzsch, Carrière, Wichura, Hildebrand, Jordan, Haeckel, Henslow, Focke and Darwin; but with the exception of Darwin, I doubt whether any one of us could say who they were, what was their training, how they worked or what they added to the world's knowledge. Presumably they were all worthy men, who, outside of the ignominy of being botanists, had nothing to their discredit; yet if they can not be termed the unknown soldiers, at least they are the unremembered soldiers, of genetics. Why? Is the growth of science essentially so slow and so continuous that our attention is attracted only by a sudden showy change, which, like the bursting of a chrysalis, is merely the sequel to something of more importance which went before? Or, does a particular piece of work, such as that of Gregor Mendel—or rather Johann Mendel, to give him his correct name, have a value *per se* which transcends the others completely? Probably both questions should have affirmative answers. I think that all too often the unknown private deserves a considererable part of the credit usually given to the colonels and generals and chiefs of staff; but in this particular case, there is evidence of a real value to Mendel's contribution which puts it in a separate class. In this array of names are competent men, who worked hard and intelligently, who made discovery after discovery; but it would have made little difference to twentieth-century genetics if they had been tailors or bricklayers instead of plant-hybridizers. I think we need not lack in respect for every one of them if we say frankly that they did not deliver the same class of goods. Mendel was familiar with the results of the earlier researches through Gärtner's huge compendium of investigations on plant hybrids, and he had read both Nägeli's paper on hybridization and Wichura's account of inheritance in the willows which appeared in the same year as his own work. There is no evidence of his knowledge of the investigations of the French horticulturists of the period, Vilmorin, Carrière, Godron and Naudin; but had he known them thoroughly, he would have been under no necessity of modifying the statement in the preface of his paper, wherein is shown more clearly perhaps than in any other place his grasp of the essential requirements of science. He says:

> Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of the hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations.

Morphology, or rather the part of morphology concerned with cell development and in particular with germ-cell development, was not in the same case as experimental breeding. The cell theory had not only one great paper in the latter half of the nineteenth century—it had fifty, each of which gave real insight into the subject. In fact, by 1895, knowledge of the cell was almost as far advanced as it is to-day. It is doubtful whether even the facts which flooded from the pedigree-culture work between 1902 and 1912 were either so numerous or so valuable to general biological progress as were those discovered by cytologists between 1877 and 1887. The mere mention of names like Van Beneden, Carnoy, Fleming, Oskar Hertwig, Strasburger and Boveri, is sufficient to call to mind what master craftsman flourished in those days. No man could take *their* papers and point out that they had failed to avail themselves of the possibilities of the method of attack used. In so far as the method had possibilities, they were turned to account.

Of demographic mathematics, the third type of work useful to genetics directly, less can be said. On the purely mathematical side, the theory of probabilities, which, during the last two decades has been found to be of such great value in solving biological problems, had long been developed far beyond the immediate needs of biologists or their ability to apply it. Such application as it had throughout this period was largely as a means of grinding out various conclusions from human vital statistics; and the results were used by economists and by life insurance actuaries rather than by biologists. I doubt whether any attempt had been made to apply the method to the solution of fundamental biological problems before the efforts of Mendel and Galton, although Quételet did use it in certain special anthropological researches.

This being the state of affairs at the time, can one by its consideration draw any helpful conclusions as to the degree of success or failure attending the efforts of the various workers involved? Such an attempt, I believe, is not altogether hopeless or wholly worthless. It is the type of introspection which every investigator ought to turn to now and then for the good of his own researches.

Let us assume the present stage of genetic thought to have been reached by a single extended and inclusive investigation, and that the technical requirements of this investigation can be determined by the simple biblical rule of judging the tree by its fruits. Having found these requirements, we can apply them as a yardstick to the actual investigations of the past and present. Without wishing to be dogmatic in the matter, I find them to be four in number. This is on the assumption that there will be no disagreement from the conclusion that science advances most rapidly by the use of the inductive method. They are: (1) the development of worthy laboratory methods, (2) the control of extraneous variables, (3) the determination of quantitative relations between the phenomena studied, and (4) the translation of the results into useful terms. The first three requirements are self-explanatory. By the fourth, I mean to say that science must have adequate shorthand formulæ by which extensive data can be expressed concisely, and that these formulæ must in turn be easily transformed into the everyday language of perception. Mendel's own system is a good illustration. Perhaps, like Archimedes, one also needs a fulcrum on which to rest his lever. It seems to me that many a piece of investigation fails to achieve the result which might well have been expected from its general conception and execution, because it begins in the middle of a complex problem and therefore has not the proper background of knowledge to carry it through. It tries to solve the cryptogram by taking a small sample from well toward the end. It usually works out much better to begin at the beginning, or at least where the other fellow left off.

Nothing novel is presented in this particular segregation of science essentials, and probably it is not so good a division as others could devise, but I believe that by keeping even this makeshift in mind, one can see rather clearly where the various contributions under discussion belong in the general scheme of things. It also gives one the opportunity of making a fair guess as to why Mendel's paper, which was in its way a model in form, remained with uncut pages for 35 years. He himself was fond of cheering his spirits by exclaiming "Meine Zeit wird schon kommen," but unfortunately it did not come until 16 years after his death.

Let us first endeavor to visualize the trend of thought during this period. There is first the host of hybridizers. Mendel took care of them in the sentence quoted. Without a proper background of facts, they had tried to solve these genetic problems having the greatest complexity. They had gone in for generic crosses by preference, and for species crosses by compulsion. Apparently it never entered their minds to make a cross between two nearly related varieties in order to simplify the complexity of the problem. They had the simple technique necessary for the particular mode of attack used; but they made no attempt either to eliminate controllable variables, to determine precisely the relation between the facts observed, or to reduce their discoveries to a system useful for predicting the consequences of like causes.

Turn now to the work of the cytologists. Naturally, in 1865, what we now accept as the fundamental facts were practically unknown. Only 15 years before botanists had been staging a battle royal on the subject of whether Schleiden's erroneous ideas regarding fertilization were true. The literature on the female gametophyte begins with Hofmeister in 1858. Real knowledge of the male gametophyte dates from Strasburger's paper on cell-formation and cell-division in 1877. The actual cells concerned in the fertilization of the higher plants were not described clearly until 1884. And the zoologists were no more clairvoyant than the botanists. Virchow did not develop the theory of cell continuity until 1858, and his papers, as is usual with new ideas, provoked attempts at scientific sabotage for a decade after that time. Fertilization of the egg by one spermatozoon awaited the demonstration made by Oskar Hertwig in 1875. The identification of the cell nucleus as the most important vehicle of inheritance, though suggested by Haeckel in 1866, was not made until it was emphasized to the world by the independent investigations of Strasburger, Hertwig, Kölliker and Weismann in 1884; and the reduction division of the chromosomes was not shown clearly until the appearance of Boveri's work on Ascaris in 1887. The general constancy of chromosome number, their individuality in size and shape, and the details of their behavior during maturation and fertilization did not come until well within the limits of the present generation.

But one must not be blinded by this course of events. Mendel's paper shows a clear grasp of the gross facts of fertilization, and those gross facts were sufficient for his needs. Furthermore, though, the cytological details which would have made it easy for others to grasp the full significance of the paper were not available until the second decade after publication, it must be remembered that recognition of his work did not come until 15 years after these details were common knowledge. For these reasons I can not believe that it is correct to account for the peculiar neglect of Mendel's work by assuming it to be ahead of the biological *knowledge* of the time.

Let us look a little closer at the problem. Cytology has been said to be the statics, and controlled breeding the dynamics, of genetics. Perhaps there is enough truth in this analogy to show a slight difference in point of view, but even this is doubtful: they are both statical in nature, and differ most in the limitations imposed by the mode of attack. For example, if one were to take all of the facts discovered by pedigree culture work, he could infer a certain organization and mechanics of operation in the germ-cells; if, on the other hand, one were to correlate properly all the discoveries of cytology, he could draw rather accurate conclusions regarding the actual transmission of characters. Genetics could not have developed as it has without both points of view, however, for *a priori* possibilities, unless tested, have no essential value. But granting this to be the truth, even the most ardent cytologist will admit that from the broad point of view of general genetics, his calling has its defects. It has a beautiful technique; but the very fact that the laboratory methods are so refined makes it difficult to eliminate the obscuring influence of extraneous variables by the very commonplace contrivance of investigating large quantities of material under controlled conditions. Again, it is practically impossible, due to the nature of the method, to investigate material in such a way as to obtain an adequate statistical representation of the facts which will permit verifiable predictions to be made. One does not decry cytology in making this statement. Cytology deserves the highest respect. But it is necessary to point out the inherent difficulties under which the cytologist works, difficulties which make his accomplishments so much more to be acclaimed. He was in much the same predicament which one might imagine would be the plight of a group of scholarly Martians who found a stranded aeroplane out of gasoline. Using the best method under the circumstances—the cytological method—they would dissect the strange visitor carefully, and make the most minutely accurate drawings of the various parts. They would then speculate on the use of each part, and finally form a hypothesis on the value and use of the apparition as a whole. This procedure would be perfectly proper. Without it, they would probably be in a quandary to know what to do with the can of gasoline dropped overboard by the unfortunate birdman and finally found by a young Martian piscatorial expert at the bottom of a canal ten miles away. With it, the gasoline would be poured in the tank, and the hypothesis tested forthwith. Now my belief is that Mendel found the can of gasoline and by his own method of reasoning knew what to do with it. But after stealing in at night and making the apparatus run, his fellow-countrymen were not able to understand his account of the machine, because the method of dealing with it was so foreign to their own experience.

I am speaking absolutely seriously. It will not do to attribute the plight of Mendel's researches to the limited circulation of the Brünn journal, for it was received by all the various universities of Germany and by many foreign libraries. It was due to a different cause, the inability of the biological mind to adapt itself quickly to an experience it had not had before. Mendel was not really a biologist though he investigated the heredity of both plants and animals—you will remember that he worked with bees as well as peas, though the records of his experiments with the honey-makers have never been found. Biology was one of his numerous avocations, like playing chess, organizing fire brigades, running banks and fighting government taxes. He was really a physicist, and brought to one of the great problems of biology the attitude of mind and the quantitative method of attack which had been in use for some time by physicists and by astronomers, and which was just coming to be used more widely by chemists. It was an unknown language to biology, though it fulfilled the essential requirements of scientific research better than anything which had gone before; and it came to biology at a time when those who were endeavoring to investigate inheritance by means of hybridization were not prepared for their task, and thirty years before the results of the slow-going cytological method of attack had progressed so far as to permit the formulation of a well-rounded hypothesis near enough to the truth to make it possible to outline the points to be verified and to make recognizable a plan of verification. Great as was the advance in cytological genetics during the latter half of the nineteenth century one can not imagine an appreciation of the Mendelian type of work by any of the investigators. Their minds were too carefully focussed on the individual fact. Either Darwin or Galton would have seen the truth clearly; but then Darwin and Galton were amateurs who were not trammeled by professional connection with the guild of biologists.

One finds additional reasons for accepting the point of view that it was the *method* which made Mendel's paper great, and the *novelty* of the method which made it unappreciated, if he studies carefully the generalized hypotheses on the subject of heredity during the nineteenth century. Really one does not need further demonstration if he has followed genetics from the time it passed out of the larval stage in 1900, and has seen how many of us assume we are exhibiting a fine degree of super-scientific criticism instead of mere stupidity when we adopt the agnostic attitude toward novel genetic methods and newly discovered facts. But the pernicious influence of abstract theories on the mind, the seductive way in which such theories lead away from reality, is worthy of a word on its own account.

Paucity of facts did not prevent the author of yesterday from putting forth theories of heredity by the score. One has only to examine some of the huge tomes on heredity of 30 or 40 years ago—Nägeli's "Abstammungslehre" is a good instance—to realize that lack of knowledge was even an aid to the publishing business.

Nägeli was able to write whole chapters on certain subjects manifestly because he had absolutely no information on them.

Each and all, these theories were as one in using mechanical interpretations which postulated active ultra-microscopic living units endowed by their creators with various qualities. No doubt this was just and proper. Such concepts have been found useful in various branches of science, and have been retained in the current theories of heredity. I only wish to point out that many of the hypotheses, described in such fanciful detail, have been hindrances rather than helps. If each of us were asked privately to state the object of scientific hypotheses, we should probably say, "to help formulate tests by which various assumptions can be justified or refuted." But publicly, in the class-room, and in the journal, we are very likely to become enamored of a well-presented hypothesis which does not stimulate research a whit, just because we are beguiled by its plausibility. Certainly a great many of the points discussed at great length by these early geneticists do not fit any better into a scientific discussion than would Cotton Mather's disquisition on the number of angels who could deploy on the point of a pin.

Recall Darwin's provisional hypothesis of pangenesis, as he termed it, proposed in 1868. His units were the gemmules, which were being given off constantly by every cell, including the germ-cells. That was virtually all there was to it, though it was propounded as a theory of heredity. In reality it was a prop, and a very weak one, to the theory of evolution. Darwin postulated this brisk inter-cellular trade in gemmules in order to show the literal-souled biologist how acquired characters might be transmitted. On the assumption that somatic modifications are not inherited, it was unnecessary. With the latter view, the cells might just as well have been insulated from each other as thoroughly as the wires in a telephone cable. Though it be sacrilege to say it, this was not the type of production to be expected from the author of the "Origin of Species." Apparently it stimulated but one experiment, Galton's blood transfusion experiment, which we now know could have told him nothing one way or the other. It acted rather like Aaron's rod, with ink gushing out in place of water, and this effect was not for the good of science.

The most notable among the various modifications of this type of theory was that of DeVries, published in 1889. Here the corpuscles, which he called the pangens, represented potential elementary body characters rather than cell qualities, and the universe of their activity was the cell rather than the body. DeVries's theory, perhaps, was some small philosophical advance over that of Darwin, but neither was a real working hypothesis in which the possible mode of hereditary transmission was outlined in such a manner that the biological student was led to make experimental tests of the postulates involved. No doubt general evolutionary thought was somewhat clarified by their introduction into the literature of the day, but they stimulated words rather than work.

Nägeli's "Abstammungslehre," which appeared in 1884, has been credited with being the first theory of heredity endowed with qualities calculated to induce research. But was it such a theory? Nägeli proposed to distinguish two kinds of protoplasm built up of physiological units, the micellæ; the one was wholly nutritive in function and required no special architecture; the other, the idioplasm, was a structure of elaborate constitution built up from micellæ representing the potential characters of the organism. I can find nothing more in Nägeli's work, and it took him 822 pages to say this. Here was a man to whom Mendel had written in detail about his work during the years between 1866 and 1873, a man who had contributed notable papers to botany on subjects ranging from the form of the starch grain to hybridization, a man who discoursed at such length on chemistry and physics that one might suppose him to have had the greatest sympathy for the highest type of useful quantitative work; but he devotes absolutely no time or energy to discussing the one paper which might have shown him a way out of the wilderness in which he found himself. Was this science? I do not believe it was. It may have been only the garrulity of senility, it may have been philosophy, but it certainly was not science. There is no evidence whatever that it stimulated a single investigation or was the source of a single discovery. But Nägeli was no worse than the other theorists of his time. The three hypotheses mentioned are fair samples of some twenty or thirty which were proclaimed to the world during the last half of the nineteenth century. They have been cited only because they show, as nothing less concrete will show, where the unreal leads.

The obverse of the medal can be illustrated by Weismann's presentation of the subject.

In Weismann's theory, the idioplasm, or germplasm, was identified with the chromatin of the nucleus. The ultimate living unit, the biophore, was a kind of biological atom active in building up organic characters. They grouped themselves together into determinates which controlled the specialization of cells. The various determinants of an organism made up the ids contributed by past generations. The ids might be one or many; and where more than one might differ slightly among themselves, thus providing for variation within a species. The ids formed the chromosomes or idants by arrangement in a linear series.

These postulates seem simple enough and not unlike those of earlier theories, but the way Weismann reasoned in endowing his corpuscles with qualities was a distinct advance. It was made possible by his thorough knowledge of embryology in which he had previously made notable contributions.

Denying the inheritance of acquired characters, and doing much toward demolishing the fallacious logic put forth as proof at that time by adherents in the belief, Weismann outlined a very stimulating conception of heredity on this basis. The immortal germplasm was assumed to be set apart at a very early cell division and passed along unchanged to the next generation, except as the activities of the living units produced occasional changes in its constitution. A provision for accurate equational division of the chromosomes and their reduction in number at the maturation of the germ cells was thus demanded, predicted and afterward realized—though not precisely in the way he supposed—by discoveries in the field of cytology.

Weismann further accounted in part for evolution by a selective struggle between the determinants of the germ cells, and for individual development by a qualitative distribution of the determinants of those cells set apart to build up the bodies which were to act as hostelries for the immortal germplasm.

No matter what views one holds as to the precise amount of truth contained in Weismann's generalization, it is obvious that it is very different from the others mentioned. Many geneticists believe the modern theory to be the outgrowth of Weismann's ideas. Wilson says he brought "the cell theory and the evolution theory into organic connection." Morgan credits him with the basis of the present attempt to interpret heredity in terms of the cell, in that he suggested three of the principles used in this interpretation. Be this as it may, there is no doubt but that Weismann was the first to utilize all the facts at his command, and to utilize them very ingeniously, in building up a theory of heredity, which, whether true or not, had numerous points that could be tested by experiment. In my opinion, it is by this criterion of ultimate usefulness and not by any analysis of its content of reality, that its greatness should be measured.

This presentation of nineteenth century genetic work necessarily having been very sketchy, no apology need be made for summing up the points of the thesis involved. Mendel initiated a method whereby the elementary quantitative relationship between hereditary phenomena could be tested and retested, and expressed his results in an algebraic notation of greatest usefulness. He thus supplied to genetics an essential methodological requirement which it previously had lacked. The significance of his offering is now apparent; but the history of both genetic research and genetic theory show that biology was not ready for such a profound change at the time. The investigators were satisfied with defective methods because they were yielding important results, and were capable of continuing to yield important results up to a certain point; and those who theorized, not realizing the defects of the current methods of research, wandered about aimlessly in the universe of the unreal. Obviously, if one is to find a clear exposition of genetic thought anywhere, it should be in the generalized theories of heredity. Taking them in the order of their issue, there ought to be a history of the development of this thought. And it seems to me that they show clearly that previous to 1890, biology was unprepared for the quantitative method of physics and chemistry; yet this method was a prerequisite for continued progress. Let us put the matter in another way, for the sake of emphasis. The older methods of genetic research were inadequate, the breeding work because the workers did not know how to use their tools, and the cytological work because the workers lacked the proper tools. Nevertheless, they made progress. They built slowly but firmly an edifice that future generations may well admire, much as the laborers of ancient Egypt built the pyramids. Finally, there arrived the point when a man like Weismann could piece together a well-rounded theory of heredity based almost wholly on this cytological evidence, which was testable by experiment. But for the tests required a new method was necessary, and this method was not forthcoming until the discovery of Mendel's long-forgotten paper. From that time onward, genetics entered a new era.

Properly, this paper should come to an end at this point; but I can not stop without delaying a moment to pay a passing tribute to Francis Galton, even though I realize that Dr. Harris will do full justice to his memory. Galton, as mentioned before, was one of the few of Mendel's contemporaries who would have appreciated his work. He was a kindred soul to Mendel, a brilliant amateur, interested in everything; and but for a matter of mere chance, he probably would have reached the same goal. The matter of chance was the study of ancestors instead of descendants. It seems a minor point, but it turned out to be important. Thus the Fates play with mankind. Galton was a leader of thought in England; he was no novice in biology; his capacity in mathematics was unquestionably great; and he turned instinctively to experiment. If only this single slender thread had not obstructed his efforts, one can well imagine how far he would have gone. But perhaps it is all for the best. Statistical theory needed Galton's guiding hand. It would not be the same to-day had it not received the quickening touch of his genius. Peace be unto his name!

Reprinted from EUGENICS IN RACE AND STATE,
Vol. II, 1923

POPULATION IN RELATION TO AGRICULTURE

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The world is not as big as it used to be. It has shrunk tremendously in the past few years, what with 40-knot vessels, 60-mile trains, aeroplanes, wireless and other devourers of distance. It is difficult to realize the change that has taken place in our outlook on many economic problems due to this globe girdling with easy means for commercial transactions and for personal migrations. In this sense the peoples of the earth have formed what may prove to be too close a League of Nations. In the olden days a people raised its own food, manufactured its own utensils, or went without. Today one sits down to breakfast, spreads out a napkin of Irish linen, takes up a banana from Central America, follows it with cereal of Minnesota wheat sweetened with the product of Cuban cane, and ends up with a Montana lamb chop and Brazilian coffee. Earlier, whole tribes, forced out of their native plains by overcrowding, girded their loins and fared westward till they found a fair land to their liking, and took it in spite of any protest on the part of the previous owners. And, as nearly always happened, there was sooner or later a racial intermixture, which, whether good, bad, or indifferent, was as a whole somewhat uniform. Today, migration is by the single family. They go to and fro upon the earth with little hindrance, seeking places to better their personal fortunes with no thought either by their new neighbors or by themselves, as to what will be the ultimate result in the intermarrying that is sure to follow.

It is not my purpose to touch upon the racial side of this great question. I have made these statements because I wished to point out that squatters have already camped on the choice spots of the earth's surface, and those who come after will have to take the byways and hedges and the stony ground. More migration has taken place during the nineteenth century than at any time during the history of the world. There has been no blare of trumpets or flying banners, but people by the millions have flitted out of their native harbors to the uttermost parts of the earth. They have settled these uttermost parts and have increased and multiplied at a greater rate than before the flitting. And why? Largely because the rate of natural increase in their native land had become so great that there was hardly enough food to go round, and modern means of communication made the flitting easy. There is a lot of nonsense current about the modern migration. We are told that people emigrate because they are politically oppressed. In nine cases out of ten they do nothing of the kind. They emigrate because they are physically oppressed; because they do not get as many of the necessities of life as they think they have coming to them; and the prime necessity is food.

A few facts will make this clear without an extended argument.

The human race has had a long pull of many million years since it first stood up on its hind legs and began to make a bid for world supremacy; it has had a history of at least 10,000 years since it developed enough mentality to leave written records; yet in all this time the natural increase was so slow that in the year of our Lord 1800 there were only some 850 million people. Since that time, a short 100 years, the population has doubled. Ten million years, let us say, to reach a population of 850 million, 10 million and 100 years to reach a population of 1700 million. Some excuse for the present generation saying their forefathers were slow.

The world has had a continuous natural increase annually for the past century of about 0.7 per cent, and the increase at present is greater than at any time during the past. My own estimate of this current annual increase, from a careful study of all available data, is 15 million or 0.9 per cent. In other words there are 2 new Belgiums to feed just now with each additional year, and the number is increasing like a compound interest table. And my own estimate is conservative since Knibbs,[1] perhaps the greatest of our contemporary population statisticians, estimates the current increase at 1.16 per cent, or nearly 20 million a year. By his estimate the world must provide food for a new France at every biennium.

With these figures before us it is not difficult to see why there has been, is, and will be emigration from the older to the newer countries. In 1800 not a single country had reached a point where the population was pressing heavily upon subsistence in present day terms. There was pressure, it is true, but it was because of unstable political conditions, poor facilities for food storage, and lack of a cheap, efficient, rapid means of transportation.

Today the story is different. China is full to overflowing, India and Japan have passed the saturation point according to Western standards of living, but are increasing because they have never had those standards, and each individual accepts the fate of a life-long grind such as we have never known, merely to keep hunger from the door. Europe as a whole is over-populated, and I firmly believe this over-population was the main cause of the world war. The Australians living on the rim of a barren desert bowl are increasing at the rate of about 18 per thousand, a rate which their agricultural possibilities cannot stand for long. The population of Canada is increasing still more rapidly. Our own United States have reached the point where there are diminishing returns in crop production. There remains, then, only Africa and South America as colonization centers with a high degree of natural soil fertility, and these continents lie largely in the tropics where the white man cannot thrive. When they are filled, nothing remains but to push into the arctics and the deserts.

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[1] Knibbs, G. H. The problems of population, food supply and migration. Scientia 26: 485–495. 1919.

Obviously the world is filling rapidly. Fifteen million more seats at Nature's banquet table must be provided yearly. See what this means in terms of land. A careful study of the available statistics shows that by and large it takes about 2.5 acres to support each individual. Some self-supporting nations can make out with less, but only when they put all their efforts into cultivating their richest land; others with less efficient methods take much more. Taking this as the average figure, then, it is necessary to plow, plant, cultivate, or otherwise tend, some 37 million acres more land each year than was ever so treated before, if the nations of the earth are not to go hungry.

It is a curious illustration of the general tendency of people to be optimistic about everything, that even many of our most eminent economists believe this wresting of additional food from the soil is an easy task. They draw wonderful pictures of what science has done in the past few years, make roseate prophecies of similar advances in the future, and keep everyone happy with the idea that the human race has unlimited credit on Nature's bank. Never was logic more fallacious.

A little arithmetic will show us that no matter how great the heights of human genius in providing for future generations the present rate of population increase, a geometrical rate continuously compounded, cannot continue indefinitely. The total land area of the globe, 33,000 million acres, seems large, it is true; but one cannot utilize all this area for raising crops. Take out the mountains, the deserts, the undrainable swamps, in short the areas not available for agriculture, and there is left about 13,000 million acres. Of this potential world farm, some 5000 million acres are now being cared for by the hand of man. According to our previous calculation, then, the maximum population the earth can support is a little over 5000 millions. *And, here is the heart of the matter, the time when this important event would take place, at the present rate of increase, is not so far distant but that some of our grandchildren would live to see it.*

Naturally this present rate of increase, which is greater than that of the past, cannot be maintained in the future. We are near the peak of the curve because of the ability of the present generation to use the powerful tools of modern industry on *new, unexploited* land. When this reserve of virgin soil approaches an end in quantity, or, what amounts to the same thing, decreases markedly in quality, population increase must diminish.

I hold that this period is approaching rapidly, and that it is high time for every progressive nation to concentrate attention on its problems of population and agriculture.

Fundamentally, the task in agriculture is the production of the primary foods, the plants. Food animals, though perhaps necessary in any permanent system of farming, are secondary products. Let us, then, give the problems of agronomy our attention.

Considering productivity alone, the agriculturist must keep in mind the plant, the environment, and the methods of forcing the plant to get the most out of its environment. Both the present status of agriculture and its future prospects can be outlined fairly well by a glance at the facts under these headings.

The food of the human race may be divided into cereals and starchy vegetables, legumes and nuts, vegetables and fruits, meat and meat products, oils, and sugars. It has been eating these products from time immemorial. Not a single food product of first importance has been brought into cultivation within historic times. Primitive man everywhere found out that his dietary needs were rather circumscribed. He came to pretty much the same conclusions in various parts of the world even as to the proportion of protein, carbohydrates, and vitamine carriers necessary for his welfare. He wasn't aware that certain oils carried a fat soluble vitamine or that vegetables and fruits carried water soluble vitamines; but he found that these products contributed to his well being, and he forthwith used them. Now no one can predict absolute failure for future efforts to introduce new food products into general use which will supplant some of those now in use, because of easy production in quantity. But isn't it more than a guess to suppose that if savage tribes in widely separated parts of the world came to use cereals, vegetables and fruits from the same families, that if even their narcotics and their arrow poisons were related, the next few thousand years will bring no revolutionary discovery in this field? There will, perhaps, be a more general use of certain fruits and meats which are now confined to rather narrow limits, but after a rather intensive study of the edible products of the world and their wild relatives, I, for one, must remain skeptical as to probability that the future will bring us anything new of importance.

On the other hand, no one can deny that we are not now utilizing every food resource at hand. When the population pressure all over the world becomes really heavy, we shall reform. We shall not only cultivate more land but we shall cultivate it more intensively. And we shall take real advantage of the sea and its denizens. Whales, seals, sea lions, fish not now found in any markets, molluscs, and sea weeds will furnish food for millions. At the same time it does not do to be too sanguine as to the immensity of these prospects. I have recently read a cheerfully optimistic book on The World's Food Resources, by J. Russell Smith.[2] Dr. Smith appears to believe that the world can go on increasing indefinitely both in people and in happiness because of the unused food ready for the taking. The probability that there will be diminished returns in getting this food to the consumer does not interest him at all, which fact makes his very readable book more of a menace than a consolation to the uncritical layman.

Several botanists and agriculturists who admit the improbability of the domestication of new plants having great economic value, and do not overestimate the relative importance of the unused wild fauna and flora, put their trust in new varieties of plants and new breeds of animals to be evolved by the scientific breeders of the future. Here again one must be very careful not to make too great a cry over little wool. Theoretical plant breeding and animal breeding under the newer name genetics have made tremendous advances in the past twenty years. We really have some fair idea of the rate at which certain organisms vary, the type of variations which occur, and the mode by which these variations are transmitted in inheritance. This knowledge places us in a much better position than ever before for predicting practical results. Personally I believe the whole matter shapes up something like this. Useful variation in the domestic plants and animals is very rare. The prospects of increased yields and better quality of food products through straight selection of variant individuals are extremely low. The real point of attack is through hybridization; yet even here it is doubtful whether a betterment greater than from ten to twenty per cent can ever occur. The great cereals, vegetables, fruits, and domestic animals all belong to ancient species which have wild relatives that will cross together and give partially fertile hybrids whose progeny are inordinately variable. Selection from among the types thus produced probably gave the useful varieties of today; and it is undoubtedly true that by the constant labor of organized workers scientifically trained, similar procedure will give the world better strains of these products, for the one thing that our modern science has done is to teach us how to save time.

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[2] New York, Henry Holt, 1919, pp. 634.

We cannot hurry Nature to any great extent, but we can eliminate useless effort. Instead of growing large quantities of first generation hybrids and making selections among them, selective elimination is made solely in the second hybrid generation where the recombination of types is found. Instead of worrying about the supposed evil effects of inbreeding, advantage is taken of the process in fixing types. Instead of working solely for stability of characters, the vigor of hybrids is utilized. Thus agriculture benefits by pure science in the same way as engineering or chemistry: the short cuts are found and put into service.

But when all is said and done, one must remember that no new processes are involved, and the few time-saving devices now used, or in the prospect of being used, for the evolution of new forms of plant and of animal life, are not going to increase our resources by leaps and bounds. The prospective increase is relatively small. The development of plants and animals under domestication has been going on for thousands of years by these same methods and the type of labor saving device we have described will only hasten matters a little. There will be no revolution. Furthermore, let us suppose that the maximum prediction shall be realized. By the expenditure of time and money for breeding projects on a scale at present beyond the dream of the most enthusiastic propagandist, current production will have been increased by 20 per cent. Is this a consummation devoutly to be wished or not? Perhaps it is merely a vehicle for exploiting a limited store of soil fertility at a greater rate, a means of dissipating capital more rapidly.

In a word, then, the prospect of newly domesticated plants supplanting those already in cultivation is negligible, the probability of increased productiveness from new varieties of the old species is much lower than is generally supposed and is contingent upon an organization and a subsidization not to be expected under present governmental policies either here or abroad. The brightest ray of hope is an increasing return per unit of area by efficient application of the best methods of crop rotation, tillage, protection, harvesting and marketing, made possible by increasing the amount of man power used. In other words, there must be a movement back to the farms, which can come only if the returns in money, comfort and satisfaction on the farms are equivalent on the average to those obtained in other walks of life.

Such general application of scientific agronomical methods as has come about in the United States during the past generation, has yielded wonderful results; but these results are difficult to demonstrate statistically. Wheat yields by decade averages from 1866 to 1915 were, 11.9, 12.3, 12.7, 13.5 and 15.0 bushels per acre. Corn yields for the same periods were 26.1, 25.5, 23.4, 25.2 and 26.6 Other crops would show similar curves. There were slight increases in production per acre, but the percentage rise is disappointing.

Actually, the true increase in production per acre due to more efficient and intensive methods of farming is really worth while. It may be as high as 50 per cent. *But it is offset and masked by the bringing into cultivation of poorer new lands, and by the impoverishment of certain of the older lands.*

Unquestionably, the universal adoption of the best methods of crop production, storage and distribution, holds out the possibility of increasing the world's food supply enormously on the present acreage. The possibility, if all of us were supermen, might be as high as 200 per cent; the probability, since we are not, is somewhere around 50 per cent. But even so there is a counter blast to this optimistic hope that comes perilously near wrecking it entirely. I cannot see in this prospect anything but temporary expediency. The limiting factors in food production are soils and climate. *In any permanent system of agriculture the soil and the climate are the true arbiters of production.* It is all very well to hire an efficiency expert to run a coal mine, but the first requisite is to have the mine.

Let us then discuss soils. Climates will have to take care of themselves. We can do little to make or mar them.

I am sure we do not care to take up a technical treatment of soils here, although it is not an exaggeration to say it is the most important subject in the world. Soil, common black earth, is the limiting factor which shall determine, not only the number of people the world may contain, but also their comfort and therefore the trend of their civilization. The main points of our present knowledge of soils and the bearing they have on present and future productivity can be presented without recourse to technicalities, however; and this will serve our purpose.

The soil itself, the home of the plant, consists of minerals, water, gases and the dead remains of various organisms; but it is affected markedly by all sorts of living plants, from bacteria to flowering types, by animals such as earthworms, and by various forms of radiant energy. It is clear therefore that the study of soils is a complex matter necessitating the energies and the knowledge of chemists, physicists, physiologists, zoölogists and botanists; and it will undoubtedly be many years before there can be a precise classification of the activities which are mixed up in crop production. Nevertheless one can clear the field for current discussion by eliminating certain things. A thousand absolutely essential factors may be involved in producing vegetable foods, but if some of these factors are never absent, if others can be influenced but slightly, interest centers on the remainder.

Now a great many of the problems of agriculture are simply those of mutual adjustment. For example, plant growth of economic importance is limited by the natural water supply, with the exception that a small amount of land may be made available for crops by artificial irrigation; but all plants do not utilize the same amount of water, and agriculturists endeavor to fit crops to the environments they require. The same thing is true of energy requirements. One variety may suit the south, another the north; one type may fit shady ground, another may grow only in the sun. Again, it is possible to make readjustments of the natural fauna and flora of a soil so that the services performed by the lower animals and plants shall not be lacking. The soil questions of greatest importance thus boil down to those known as the problems of soil fertility.

One thing upon which all authorities agree is that natural soils differ in their productivity, and that particular methods of treatment may enhance or lessen this productiveness. Upon the proper interpretation of these acknowleged facts depends the whole future of the human race. Upon the truth as we see it today, therefore, should depend our attitude toward current agricultural policies.

The ideas of soil fertility accepted by most chemists and physicists have grown out of the theories advanced by the father of organic chemistry, Liebig. He showed that crops removed various elements from the soil. In particular, there were three, nitrogen, phosphorus and potassium, which were removed in large quantities and in which many soils might soon become deficient. His remedy was the application of compounds containing these elements to the soil. Thus he endeavored to give a chemical justification to the ages old empiric application of certain materials to the soil as fertilizers.

The Liebig theory is an exemplification of the old adage: you cannot eat your cake and have it. If you use up the natural fertility of the soil, it is gone. Four minus two is two.

Elaborations of this theory have been generally accepted. We cannot consider critically all of the reasons why it has found acceptance. Thousands of controlled experiments have been made, and with the most concise abstracts possible the discussion would run into several volumes. Suffice it to say that they all point to the truth of the following points: (1) With the exception of the three elements nitrogen, potassium and phosphorus, all ordinary soils contain practically inexhaustible quantities of the elements essential to plant life. (2) Application of nitrogen, phosphorus and potassium to soils which are deficient in them nearly always give increased plant growth. (3) Application of a single one of these elements to soils where that element alone is deficient, gives a similar increase. (4) Application of one or more of these elements to soils in which chemical analysis shows there is no deficiency, results in little if any increased growth.

It seems to me no extended argument on these points is necessary. Plants will not grow without nitrogen, phosphorus and potassium, and these three elements exist in very limited quantities in most soils. The amounts removed by the plants can be calculated with great precision, and the amounts lost through leaching can be estimated with a fair degree of accuracy. The result, by the arithmetic commonly in use, is to find that the majority of soils cropped intensively without fertility replacement have a "life" ranging from a few generations to a few hundred years. Of course one can not calculate a period at which a soil will become absolutely unproductive, for the crops merely diminish at a more or less constant ratio, but one cannot only calculate, he can actually point to experiments of the Rothamstead and of the Illinois, the Ohio and other agricultural experiment stations, where continuous cropping for a quarter of a century without rotation or the use of fertilizers has brought the crop return to a vanishing point so far as economic profit is concerned.

Unfortunately too many people fail to realize the gravity of this situation. They feel that since the world has "carried on" in the past, it will "worry through" in the future. There is a *laissez faire* policy in agriculture as well as in politics. They argue that as numerous soils in Europe show an increasing productivity after "thousands of years of cropping," as the use of fertilizers is comparatively recent since the German potash deposits were only opened in 1862, and the composition of these soils compares favorably with that of rich soils in the United States, it is impossible to maintain that soils wear out. One might well rest the defence on common knowledge of arithmetic, as before, but there are some concrete statistical fallacies in this argument which should not be passed over in silence.

In the first place it should be realized that Europe has not been cropped to death for thousands of years to support an exceedingly numerous population, as is supposed by certain writers. A careful examination of the available statistics of population in England, France and Germany, leads to the conclusion that Europe as a whole has just about *tripled* in population during the last century. Europe, then, is only now being cropped to death; her soils have not really been tested out for centuries. Further, the statistics of crops cited are evidently from particular farms. Only very up-to-date efficient plantations would keep records for decade after decade. Whether they came from especially rich farms or not, any increase in crops could undoubtedly be attributed to a gradual infusion of modern methods of tillage, crop rotation and machinery. And if this be not enough to account for the results, one can turn to the use of fertilizers, for although the use of pure chemicals is modern, the use of stable manure on crops is probably as old as husbandry. Do you recall Streeter's "Dere Mable" books? In one place he says: "You can tell how rich a Frenchman is by the size of his manure pile. The bigger the pile the bigger the man you are in your home town. All I can say is, 'I'm glad the people we live with are poor. I'd hate to be billeted with the Mayor.' "

This is not only France, it is all Europe. Only by painstaking conservation of the better soils has Europe managed to come as near to feeding her population as she has, and still she is on the wrong side of import-export ledger by millions and millions of tons annually. We have not reached the point where necessity brings such careful husbanding of resources. We are the prodigal heirs; nevertheless the day of accounting is on the calendar.

We have a comparatively new, supposedly inexhaustible country here. Its problems of population and their sustenance may be presumed to be light and easy as yet compared with those of some of the older parts of the world. But the problems are the same in type. We are approaching those already met by the Chinese; are we confronting those which will be met later by the colonizers of Africa and South America? If we turn our attention to our own problems, therefore, we will view in miniature those of the world; and in addition there will be something of a personal interest in the matter.

Since we began to take the census in 1790, a short space of 120 years as history goes, we have expanded from a floor space of 200 million acres and a population of 4 million to one of 1903 million acres inhabited by nearly 110 million people. The genius of the American people for building up their homeland has made things progress nearly as rapidly as our childhood friend the genius of Aladdin, the slave of the lamp. The land has been spanned by a network of railroads. Cities have appeared as if by magic. Industry has expanded by leaps and bounds. Wealth has been at hand for the taking by any resolute character with the courage of his convictions. Just such men were our forefathers, and they prospered beyond the dreams of Midas. And under the governmental policy of encouraging individuality who could say them nay? Yet this policy of "each man for himself" with no plan for the conservation of natural resources, may result in the metaphorical fulfillment of the remainder of the proverb, and "the Devil will take us all." Surely no thoughtful man now denies that there has been a tremendous waste of coal and oil, of the metals and the timber, and last but not least of soil fertility. We have had a master passion to expand as rapidly as possible, without thinking that we were emulating the prodigal son in running through his patrimony. But prodigals we were and are.

It is curious what false ideas in regard to the remaining agricultural possibilities of the United States are held by so many people. They seem to feel that the country is a kind of a Ceres' cornucopia with no bottom at all—a never ending supply of the fruits of the earth. Not long ago I asked a well trained business man how much more land he thought could be brought in cultivation. His off-hand estimate was between 400 and 600 per cent—a rather astounding guess.

As a matter of fact half of our land total of 1903 million acres is now in farms, though it has not paid thus far to improve and cultivate all of it. There remains a reserve of over a billion acres, it is true; but nearly half of this is arid land having a precipitation of less than 15 inches, and less than 10 per cent of that will become available after the completion of all irrigation projects possible under the present system of construction. When one adds to this a proper allowance for permanent forests, for unusable swamps, and for cities, roads and railroads, there are something like three-quarters of a billion acres which must be forever withheld from agricultural use. There is left 300 million acres, roughly 35 per cent of our present farm lands, which may be incorporated with them. Thirty-five per cent is some reduction from the 600 per cent estimate, isn't it? And in this connection there is another matter where one is likely to go wrong. There are no rich new valleys and fertile virgin plains to be brought under the plow when the need arises. This 35 per cent is the poor expanse of waste passed by as worthless by the farmers of today and yesterday. It will be cultivated sometime when our descendants come to that point of subsistence pressure, but not until the half of the land now in farms but used as woodlots and pastures, has been made to yield to the plow and harrow, and not until food prices reach a point where the contemporary growlers about profiteering look back at the prices of 1920 with eyes of envy.

But let us see what we are doing with the land we are using: Is there any method of showing whether the returns are increasing or diminishing? It is a difficult question to decide because of the complications involved, but personally I believe there is conclusive evidence that the output per man is diminishing, despite the improved varieties of the staple crops and the new machinery for tilling them, despite the more general adoption of crop rotations and pest control measures. It is only natural that this should be so. It is the general history of every country. The cream is skimmed off during the early years of cultivation by extensive methods. The more intensive methods prevail; and the yield per acre increases, while the yield per man decreases.

There was a great expansion of farming here during the seventies and eighties. The rich lands of the middle west were *cultivated extensively.* Low yields per acre were obtained because of these methods, but the number of new acres utilized was so great that overproduction and extremely low prices resulted. More recently there has been a trend toward intensive farming, hence an increased yield per acre and a lowering of the production per man. For example, the average yield of all crops between 1908 and 1918 was about 15 per cent greater than that between 1880 and 1890, but it took a considerably greater expenditure of man power per acre to get this yield. Those who point out that we have not reached the yield per acre of the best farmers of Europe would do well to remember this point. The most intensive farmers of Europe, the Belgians, cultivate about 5 acres per man; in the United States the comparative figure is 26 acres. We shall come to the European figures of production on a part of our area in time, but at the cost of more toil than is expended on a unit area today. This tendency is perhaps most clearly seen by comparing the increase in population in the decade 1900 to 1910 with the increase in farm lands. The first was 21 per cent, the second only 4.9 per cent, yet the people were fed. Furthermore, see what the farmers did in 1919 with very few extra men, merely by working harder themselves, when there was an incentive in extraordinarily high prices. The acreage of cereals alone was 33 millions greater than the average from 1910 to 1914. But it is also clear that these matters are determined by the economic laws which do actually prevail—prices controlled by supply and demand. What we wish to talk about is another thing.

If a multimillionaire shoe manufacturer should throw millions of pairs of shoes on the market at less than the cost of production, the prices of the shoes of other manufacturers would be forced down in consequence. The fact that the altruist of the shoe trade was using up his capital would not affect the response of the shoe market. Similarly farm prices and gross production are responsive to the same economic laws. Whether the farmer is using up or conserving his soil fertility capital, makes not the slightest difference. But it does make a difference to the future of the country.

Now the United States is not really a food exporting country. Normally we are now consuming what we produce. In the years 1911–1914, the total average export of foods was 52,746 metric tons less than the imports, yet for the four war years the annual average export balance was 3,169,495 metric tons. The United States was again made a great exporting nation during the war by high prices. The people did not eat less food except in rare cases. Pearl[3] has shown that the per capita food intake continued about as high during the war as it was immediately before. They wasted a little less, as is shown by the garbage collection figures, and they changed their diet somewhat to meet the requests of the Food Administration; but they not only ate as much food per capita as before, they demanded it in the most expensive form. The exportation was possible because the annual food production in kilograms per capita increased from 888.7 for the three pre-war years, to 918.5 for the war period. And this was wholly an increase due to price stimulus.

Now when we forget about the war and go back over the *production figures per capita* from 1870 to 1916, we find that meat has decreased markedly and total food production slightly. We are still getting plenty to eat, but overproduction and cheap food have stopped. This is as it should be. What I am concerned about is this. The time for easy food production from virgin lands has vanished. Fertilizer consumption has increased by leaps and bounds. Some farms have been abandoned through depletion to the point of exhaustion, though it is true abandonment is largely for other reasons. New lands less productive in nature are gradually being put under the plow because prices are such that they yield a fair return. Intensified farming is increasing. In all this there is every evidence of diminishing returns in agriculture, yet few take any thought of what it means.

This is what it means. The farmers of the past century received in trust the agricultural capital of the country. The people gave it to them without asking them how they would use it. These trustees have drawn on the capital, and they have done this because they were forced to do it. They were told, in effect: "We have given you this land for nothing. Don't treat it as if it were the capital assets of a big business. Go to it and produce us food, and we will pay you just the wages of your labor for it. Take no account of the depreciation." This is bad bookkeeping. It is ill use of the Nation's resources. But it has not been the fault of the farmer. He has done just what he was forced to do. The result is that the potential, and in many cases the actual producing power of the soil, has been diminished. We have fared well by taking no account of how our children will fare. We are coming to a point, however, where diminishing returns will become more and more in evidence. Perhaps then we shall do something to counteract our previous neglect. If we do not do it, if we continue a *laissez faire* policy, we shall continue to fare well for a time and then the pressure will become serious in a very short time. There will come the day of reckoning. If we do have the forethought to inaugurate a permanent progressive agricultural policy, we shall have to pay an immediate premium on our future insurance, but will it not be worth it?

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[3] Pearl, Raymond. The Nation's Food. Saunders, Phil. 1920, pp. 274.

You no doubt will ask what I have to suggest? There are many sound proposals that one could make, but our time is too limited to do more than to sketch them roughly. And besides no one could present proposals satisfactory in detail without much more careful study than I have been able to give the matter.

Perhaps the most helpful means of putting agriculture on a sounder basis is an indirect one—severe permanent restrictions on immigration and possibly temporary prohibition. Since 1820, 28 million immigrants have arrived at our shores, and at the last census only 53.8 per cent of the population were native whites, born of native white parents. These newcomers have been welcomed and possess all the rights and privileges of the oldest inhabitants, yet it is wholly proper for those on the grounds to forbid further entrance if that be necessary. Is it not necessary? Any present cry for immigration can only be made by fools, hypocrites or ignoramuses. We have grown too fast already and have not moulded our population into a single nation. Those who call for immigrants wish to exploit their labor at low wages, not realizing that cheap labor is generally the most expensive in the long run. Our later immigrants have found their ways largely to the cities where they have been engulfed in the maw of the factory, giving the farmers still more mouths to feed besides tempting the native farmer boys away by seemingly larger wages to be their superintendents. An increased number of mouths to feed does not solve the food production problem, particularly when many of the owners of these mouths are hardly productive enough to earn their keep. The only solution of this predicament, from a purely business point of view, is to pay the immigrant the starvation wage which the pro-immigration propagandist hopes to pay him. But fundamentally this is neither good business policy nor good ethics. It is no doubt the memory of short periods of such treatment, that makes many aliens who may now be receiving more than they really earn, lend ear to any pernicious radical propagandist with an ax to grind. No! If we are to settle this great economic problem, we must not only cut down this too rapid expansion of population through immigration, but should cut the native birthrate as well—if it be done in the right place.

There should be no misunderstanding of the wise position to take on this phase of the problem. Certain transportation companies have always been willing to betray the country into accepting undesirables for the thirty pieces of silver involved in a steerage passage. There are those whose love of race is stronger than their love of country, hoping to transplant their kindred root and branch to this more fertile garden spot. And there are the pure exploiters. With their wiles and intrigues we cannot deal here. But in addition there are numerous dabblers in sociology who honestly believe immigration should be encouraged for the twofold good of the immigrant and of the country. To such as these one may say: "Do you realize that emigration from a populous country relieves but a few individuals, and that there is an immediate increase in the birthrate which soon restabilizes the economic pressure? Do you realize that the immigrant always comes with a lower standard of living, and that because of his individual inability to produce enough to reach the new standard which he meets, he tends to lower it by his competition? Do your realize that the unrestrained fertility of the new arrivals depresses the fecundity of the native sons and daughters who know that the time of diminishing returns has come? Do you realize that only a comparatively small natural increase in population is possible for this country if there is to be time for the economic readjustment required by diminishing returns?" If he is thoughtful, if he is open minded, if he is really worthy his place in the State, the immigration policy of the future will be solved.

Second, there must be a reorganization of what is already being done for the farmer by means of the United States Department of Agriculture and the State Experiment Stations. I do not mean by reorganization a radical change in present policies, for these institutions are doing wonders. There might well be some greater attention paid to pure science, since if no heed is given to increase of knowledge, diffusion of knowledge is hampered. What I meant was an expanded investigational and pedagogical aid to agriculture reorganized financially. There ought to be a lot of $20,000 men in this work, and they ought to get $20,000. Even if there were enough high class men to be found to occupy these places at starvation wages, it is beneath this country to demand it.

Next the farmer's financial way should be made as easy as possible. Farming of the right sort should be made attractive. Something is now being done in the way of extending credit. This will help matters, if properly handled. But the kind of operation carried out by the Farm Loan Banks does not really get to the bottom of things. The true financial worry of the farmer comes from having to plant his maximum acreage from six months to a year before he receives his returns, without having any idea of the price he is to receive for his labor. He not only has to plant, but he has to plant pretty much the same crops as he planted the previous year, for proper farming means specialization. He is therefore between the upper and the nether millstones.

Now I am sure I can give no concrete remedy for this problem. It is too big and involved for off-hand solution. Yet it must have a solution, even though it be somewhat imperfect, if the nation is going to make the most of its resources. Solutions should be worked out by experts and Congress forced into line to try them out. Something can certainly be done to give the farmer a return for his products that is based on the cost of production, as in any other business; and that is all he asks.

As a beginning it would seem to be possible to increase the facilities for distribution, and to decrease the margin of profit between the producer and ultimate consumer. There are necessary and legitimate charges for the transportation, storage, manufacture and retail marketing of food. Whether the profits made by those who undertake these tasks are unreasonable or not, is not at all clear from the available statistics. It is clear that the methods in use are unscientific and wasteful to an absurd degree, that they are needlessly involved and complicated, and that they are practically free from governmental supervision. The result is a price fluctuation governed by supply and demand which works to the detriment of the public good. The farmer is affected first by any fall in prices through overproduction. He forthwith curtails too much, benefits a little by increased prices, and forces great hardship on the ultimate consumer. Any breakdown in the chain between the farmer and the consumer also reacts sharply and quickly against the latter, though the reaction ultimately reaches the producer.

The mechanism by which this muddle is to be clarified is for the future to reveal. It may be built up by government supervision, it may be evolved through the initiation of active coöperative marketing and buying; but whatever the general means to the end, the problem is one having several sharply defined phases:

1. Accurate and comprehensive agricultural statistics should be collected and made available quickly. With these facts as a basis the government should be able to keep its hand on the throttle of agricultural production, and should be able to prevent serious excess or deficiency in each crop subdivision. By similar advisory work, it should gradually be possible to have the food resources of the nation marketed more nearly continuously throughout the year. Both glut and scarcity might thus be avoided.

2. Transportation should be facilitated in every way consistent with industrial economy.

3. An analysis of the means and the costs by which food passes from producer to consumer should be made, with a system for more direct marketing and lessened middleman costs as the outcome. A markedly

successful effort toward this end was made by the United States Food Administration during the war, so it is not a mere dream.

4. Deliberate speculative manipulation of food prices should be prohibited.

5. Hazards of buying and marketing on short-time contracts which are necessarily attended by somewhat of the speculative should be reduced to a minimum by whatever means is found adequate.

6. An agricultural foreign policy should be inaugurated which will have as its primary aim the stabilization of prices and the conservation of soil wealth.

With even a semblance of a solution to these important questions[4] we shall have what we have not had before—an agricultural policy encouraging a continuous normal food production per capita with price fluctuations reduced to a minimum, simply through giving the farmer a square deal.

The final suggestion is forecasted by the arguments set forth earlier. In last analysis the future food supply of the world depends upon the conservation of soil fertility. Our soil fertility is the greatest single asset of the nation, and it should not be permitted to be dissipated. The campaign for a truly permanent system of agriculture where productivity is kept high without soil robbery should not be allowed to lapse, but should be prosecuted more and more vigorously. Through proper rotation of crops, the return of waste products to the soil, and the use of our comparatively unlimited supply of fertilizers, this may be done. We do not want to wait until necessity presses us to adopt some of the methods of the Chinese.

Listen to what King,[5] the only soil expert of the United States—so far as I know—who studied the subject on the spot, says of Chinese and Japanese practice:

"In China enormous quantities of canal mud are applied to the fields, sometimes at the rate of 70 tons per acre." It may be said in passing that China has 200,000 miles of canals built for agricultural as well as for purely commercial purposes. "So, too, where there are no canals, both the soil and subsoil are carried into the villages and there between the intervals when they are needed they are, at the expense of great labor, composted with organic refuse and often afterwards dried and pulverized before being carried back and used on the fields as homemade fertilizers. Manure of all kinds, human and animal, is religiously saved and applied to the fields in a manner which secures an efficiency far above our own practices.

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[4] Cf. Hoover, H. C. Some notes on agricultural readjustment and the high cost of living. Sat. Eve. Post, April 10, 1920.

[5] King, F. H. Farmers of forty centuries. Mad. pri. pri., 1911, pp. 441.

"Statistics obtained through the Bureau of Agriculture, Japan, place the amount of human waste in that country in 1908 at 23,950,295 tons, or 1.75 tons per acre of their cultivated land. Japan's production of fertilizing material, regularly prepared and applied to the land annually, amounts to more than 4.5 tons per acre of cultivated field exclusive of the commercial fertilizers purchased."

I leave this quotation with you as a parting word, to be remembered, I hope, as a real lesson, which teaches that forethought now is the only method of assuring an adequate future food supply.

GENETICS OF BUNT RESISTANCE IN WHEAT

BY

E. F. GAINES

Reprint from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XXIII. No. 6 : : Washington. D. C. February 10, 1923

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE,
WITH THE COOPERATION OF THE
ASSOCIATION OF LAND-GRANT COLLEGES

GENETICS OF BUNT RESISTANCE IN WHEAT[1]

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INTRODUCTION

The losses due to bunt, *Tilletia tritici* (Bjerk.) Wint., in the Pacific Northwest have been steadily increasing for the past 25 years, notwithstanding the most earnest efforts on the part of scientists and farmers to reduce them. The seed has practically always been treated with blue vitriol or formaldehyde, but in spite of every precaution the winter wheat often contains from 10 to 50 per cent of bunted heads at harvest time, apparently due to soil infection from wind-borne spores. In the State of Washington alone the most conservative estimates place the losses at more than 1,000,000 bushels of wheat annually. The bunt problem has caused, and is causing, more interest and anxiety than any other of like nature through the winter wheat districts. Spring-sown wheat seldom produces a bunted crop if the seed has been carefully disinfected, the spores in the soil having perished during the winter.

The work has been closely linked with the other cereal and pathological investigations at the Washington Agricultural Experiment Station, and many friends and coworkers have contributed to the material in hand. Acknowledgment is here made to the various members of the staffs of the Washington Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for their ever-ready help and enthusiasm; to E. M. East for helpful suggestions and criticism of the manuscript, and also to H. B. Humphrey, H. M. Woolman, E. G. Schafer, and F. D. Heald for suggestions and corrections in the presentation of the subject matter.

When the writer became assistant cerealist of the Washington Agricultural Station in 1911, many inquiries were coming in from farmers concerning control measures for bunt in winter wheat. The limitations of seed treatment were well known, and soil sterilization seemed impracticable under the extensive system of wheat raising on the great farms of the winter-wheat belt. The possibility of obtaining or developing a strain that would resist the attacks of the fungus and at the same time fulfill the requirements of winter hardiness, prolificacy, milling quality, etc., seemed the most promising solution of the problem. Accordingly hundreds of varieties from the principal wheat districts of the world have been introduced and many hybrids have been made in an attempt to find or develop such a wheat. Preliminary reports (*13, 14*)[2] of this work have already been published.

The following paper deals with that part of the investigation concerning the comparative resistance of different wheats and the inheritance of the factors that cause resistance, as expressed in the hybrid segregates of succeeding generations.

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[1] Accepted for publication August 31, 1921. Submitted to the faculty of the Bussey Institution of Harvard University in partial fulfillment of the requirements for the degree of doctor of science, April 29, 1921.

[2] Reference is made by number (italic) to "Literature cited," p. 476-479.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XXIII, No. 6
Feb. 10, 1923
Key No. Wash.-1

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RESISTANCE PHENOMENA IN GENERAL

RELATIONSHIPS AND RESPONSES OF HOST AND PARASITE

It has long been known that plants and animals vary greatly in their susceptibility to disease, and that the causal organisms are more or less restricted in their choice of host. This variation may be attributable to the inability of the causal organism to set up a pathogenic relationship with the host or to its inability to function after having established such relationship. Failure to infect may be due to an incompatible optimum of temperature, moisture, light, or nutrient materials. These factors may also provide the physiological basis for failure to function after infection has taken place. In addition, the outer coat may offer a mechanical obstruction. Thus, the bark of trees, the seed coats of grain, and the skins of animals offer a powerful protection against invading organisms which gain a ready access if injury occurs. However, a great majority of the pathogens that have been studied experimentally seem to have less difficulty in gaining entrance to closely related resistant forms than in continuing that existence after having gained entrance. Thus in Marryat's experiments (*32*) infection by yellow or stripe rust [*Puccinia glumarum* (Schm.) Erikss. and Henn.] occurred with equal readiness through the stomata of resistant and susceptible wheats. The susceptible Michigan Bronze wheat seemed to nurture the infection tubes and hyphae, the cells accepted the haustoria without shrinking, and normal spores were soon produced. Hyphae never seemed to flourish in the resistant Einkorn, but appeared stunted and watery and seldom produced haustoria. They soon died, but in dying they killed the host cells with which they came in contact—appearing to be mutually toxic. The American Club, intermediate in resistance, sometimes killed the fungus without reducing the vigor of the host cells, but in other places the hyphae were found flourishing while the host cells seemed to be dying. When spores did develop there was not enough force to break the epidermis. Apparently resistance in this case was produced by antitoxins furnished by the host and toxins furnished by the parasite. Ward (*60*), also working on the histology of resistance, showed that size of stomata, hairs, or comparative leaf surface had no influence whatever on the susceptibility of different bromes to brown rust, *Puccinia dispersa* Erikss. and Henn. After treating a mass of data statistically he drew the following conclusions:

> Resistance—is not to be referred to observable anatomical or structural peculiarities but to internal, i. e., intraprotoplasmic, properties beyond the reach of the microscope, and similar in their nature to those which bring about the essential differences between species and varieties themselves.

This was in 1902. Since then a great mass of experimental work in plant pathology, bacteriology, and medicine has added weight to his conclusions. For example, Stakman (*45*), who has done much work on the phenomena of resistance in cereals and grasses to the stemrust, *Puccinia graminis* Pers., concludes from his histological study of hyphal invasion (to quote his summary):

> 1. When plants practically immune to *Puccinia graminis* are inoculated, the fungus gains entrance in a perfectly normal manner.
> 2. After entrance the fungus rapidly kills a limited number of the plants cells.
> 3. The fungus, after having killed the host cells in its immediate vicinity, seems unable to develop further.
> 4. The relations between plant and parasite in partially resistant and almost wholly immune plants are different in degree only.

5. Hypersensitiveness of the host seems to be a common phenomenon not only among plants somewhat resistant to *P. graminis* but also among those almost totally immune to it.

These conclusions point to the probability that resistant and susceptible plants differ in the chemical composition of their cell walls or protoplasm or both. Valuable information might be gained along these lines by such investigations as the recent work of Rumbold (*43*) and Thiel and Weiss (*55*). Rumbold killed Chestnut trees by injecting an extract of the cankerous tissue caused by the chestnut-blight fungus [*Endothia parasitica* (Murr.) P. J. and H. W. Anderson] into the trunks of healthy trees. Extracts from healthy tissue produced no effect.

Thiel and Weiss were able to germinate teliospores of *Puccinia graminis tritici* Erikss. and Henn. in midwinter by soaking them 15 minutes in 1 per cent citric acid, although untreated spores would not germinate at all until spring. Other acids, lipoid solvents, and sodium hydroxid were ineffective. From this it would seem that the acid had acted as a specific activator through the cell wall on the protoplasm. Susceptible hosts may contain similar activators which stimulate the metabolism of the parasite.

The delicate chemotactic balance between host and parasite as an explanation of the differing degrees of resistance finds support in the investigations of Spinks (*44*) who found that nitrates, and especially lead and zinc nitrate, increased the susceptibility of wheat and barley to mildew and rust. Furthermore, potash and lithium salts decreased susceptibility; but resistant varieties maintained their relative resistance under either condition.

From this it is logical to assume that species or races which vary in their resistance to disease-causing organisms when growing under identical environmental conditions do so because of their physical and chemical individualism. If this be true, then the complex of causes responsible for resistance and immunity in plants is very similar to, if not identical with, those producing resistance and immunity in animals.

The classic researches the last 25 years in the antitoxin laboratories have shown that susceptibility to disease is caused by the physicochemical reactions of poisons, toxins, ptomains, etc., produced by the causal organism in the various tissues of the host. In acquired immunity the host cells have the power to develop counteracting chemicals which neutralize the harmful substances produced by the pathogene. In natural immunity either the counteracting chemicals are already present, the temperature, light, food, etc., are not optimum for the pathogene, or else the composition of the protoplasm of the host is such that no chemical reaction takes place. An illustration of the latter condition is described by Zinsser (*62*) regarding tetanus from the work of Blumenthal (*6, p. 185*). As is well known, the central nervous system is the special point of attack for tetanus poison, and Blumenthal found that tetanus toxin was neutralized by the brain tissue of susceptible animals, but the brain tissue of resistant animals like the chicken had little or no neutralizing power. The frog is also immune to tetanus under normal conditions. When the toxin is injected, the body temperature being too low to allow the combination between toxin and nervous tissue to take place, no tetanus results. If, however, the frog is placed in a tank of warm water and kept at the normal temperature of the human body it succumbs to the effects of the toxin. Clearly chickens and frogs are resistant to tetanus for very different reasons. The first, because of the different chemical compositions of the brain; the second, because of physical incompatability to the parasite.

Zinsser in discussing nonheritable natural immunity, says:

> The individual differences in resistance which unquestionably exist among members of the same species and races are very difficult to explain, but, as far as we can tell anything about them at all, they seem to depend upon variation in what is popularly spoken of as "general condition."

This indicates that the counteracting chemicals are present in greater or less amounts even in organisms that readily succumb to a given disease. Or, stating it in another way, the particular tissue to be attacked can suffer injury and recover if the host is well nourished and in an optimum environment for its existence. This phase of natural immunity leads directly into the phenomenon of acquired immunity.

Injuries or wounds in plant and animal life not only stimulate repair but also growth and rejuvenescence. If the injury is sufficiently severe, or repeated often enough, the energy required to stimulate the affected part is too great a tax and the organism dies. Any factor which reduces the energy of the organism, such as fatigue, malnutrition, unfavorable temperature, etc., should reduce its resistance to disease, and this is what actually happens.

In naturally acquired immunity, the injury caused by the toxin of the invading organism stimulates an overproduction of the material used up in neutralizing it, so that subsequently more and more of the toxin can be neutralized without fatal results. Similarly in induced immunity, the injection of increasing amounts of toxin can be borne owing to the preponderantly greater amounts of neutralizing substances elaborated. In immunization work, one of the standard methods is to inject sublethal doses of fully virulent organisms in order to stimulate this great overproduction of these counteracting chemicals, which are cast into the blood and neutralize the counteracting poison at once. Such immunized animals may or may not, depending upon the particular antigen or toxin, retain this immunity for life.

Since the beginning of modern medical sceince it has been known that people having once recovered from such diseases as plague, cholera, smallpox, or yellow fever will not again during their lifetime contract it; but lasting immunity is not conferred by one attack of such diseases as pneumonia, tetanus, or influenza. In attempting to explain or make use of these phenomena a great branch of physiological chemistry has been developed.

The most comprehensive attempt to explain the causes of immunity is that offered by Ehrlich (*10*), in which he conceives the cells of host and parasite as aggregations of complex molecules which are themselves complex. Complex molecules react with one another through certain of their side chains, but only when these side chains have a certain definite correspondence in structure. The reactions of immunity represent only a repitition of the processes of normal metabolism. A receptor is an outlying part (the chain) of the cell which is able to combine, by means of a so-called haptophorous group, with foreign (protein) molecules. The haptophorous group of the receptor is able to combine with a food molecule or with a toxin molecule. Such combination stimulates the cell to produce more receptors which, in the case of toxin combination, may result in over production, the superfluous receptors becoming detached from the cell. These are called amboceptors because they have two haptophorous groups, and in the immunizing process they act as links to bind the invading cells to the complements which are normally present and which, when so united to the foreign cells, are able to destroy them by means of a zymotoxic or toxophore group. The amboceptor with its complement constitutes a cytotoxin, hemolysin, or bacteriolysin. Other detached receptors act as antitoxins, as agglutinins, or as precipitins. Toxins have a haptophorous group by which they combine with antitoxins, and a toxophorous group to which their injurious effects are due. A toxin which has lost its toxophorous group, as by heating, is called a toxoid. A complement which has lost its zymotoxic group is a complementoid and an amboceptor that has lost one of its haptophorous groups is called an amboceptoid. The presence of a foreign cytotoxin leads to the presence of an anticytotoxin, which may act on the amboceptor or on the complement.

Whether Ehrlich's or similar theories can be extended to account for all the complex phenomena of reistance and immunity in plants and animals remains for the future to decide. Like the hypotheses in physics and chemistry, it offers a shorthand method for explaning the facts. It helps to make a clearer understanding of the phenomena of both natural and acquired resistance and immunity and also explains in a logical way the change in the virulency of organisms, in so far as there is any explanation at present. For example, as Walker (*59, p. 34*) has shown the pathogenic organisms themselves may be immunized against immune serums by cultivating them in media containing increasing portions of the immune serums.

It is not too much to suppose, therefore, that pathogenic organisms themselves are subject to contagious diseases, suffer from malnutrition, and benefit by becoming acclimated, in response to the same laws that operate in higher organisms. Changes in virulency can probably be explained as the interaction of these factors in the majority of cases; but the plasticity or heterozygous character of some forms offer the possibility of genetical change by selection. The brief time required for each generation in many of the lower forms and the large numbers produced would make this a quick and permanent method of adapting an organism to its environment. Since, in general, the lower forms are more constant or stable than the higher forms, it is probable that such genetic change by selection is very limited.

From the foregoing discussion it seems that disease-resistance phenomena in both plants and animals are due to the same general causes. The hosts of a given parasite may vary both morphologically and physiologically; and according to their physico-chemical complex react diversely in their irritability and response to such factors as temperature, moisture, light, nutrition, and poison. The same organism will react differently to the same stimuli at different stages in its development. In so far as the subject has been studied, the parasite or disease-causing organism seems to vary also, and according to the same laws that govern the host. Added to this, the host is changed within certain limits, chemically, by its food supply and other external stimuli, such as other diseases or mechanical injury. The parasite probably has its diseases and is no doubt changed chemically by them and also by its changing food supply. In the delicate balance between these two organisms, host and parasite, there is a physico-chemcial complex so intricate that it has thus far been impossible to comprehend or to analyze it in all its detail. Heritable resistance represents a permanent upsetting of this balance in favor of the host, caused by the variation in its physico-chemical complex.

INHERITANCE OF RESISTANCE

Acquired resistance and induced immunity, like other acquired characters, have not been shown experimentally to be inherited in the ordinary sense of the term, but each generation must recover from a given disease or be vaccinated (or inoculated with the proper virus) in order to acquire immunity. Inherent or natural resistance is definitely passed on from generation to generation like morphological characters. When such a resistant race is crossed on a susceptible one, resistant and susceptible races may be isolated in subsequent generations, which show that the same laws of inheritance which control other characteristics are in operation in determining the causes of resistance. Biffn (*2*), in England, found resistance to yellow rust *Puccinia glumarum* Erikss. and Henn. and resistance to mildew *Erysiphe graminis* DC. in barley to be a simple mendelian recessive. One of his resistant hybrid wheats has become of commercial importance and has retained its resistance at Cambridge for 16 generations. Nilsson-Ehle (*34*), in Sweden, corroborated Biffen's findings as to the heritability of resistance to yellow rust, but in his crosses he obtained segregates more resistant and more susceptible than the parents, which he interpreted as being due to modifying multiple factors. Hayes, Parker, and Kurtzweil (*15*), in America, found the same phenomenon in connection with resistance to stemrust *Puccinia graminis* Pers. in wheat and in addition found linkage between resistance and certain durum and emmer-like characters.

Other genera that have been studied genetically in regard to resistance to various diseases, in general show the same type of inheritance. McRostie (*30*) ,working with two independent strains of the bean anthracnose organism *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., showed resistance to anthracnose to be dominant to susceptibility. When a bean resistant to both strains was crossed on one susceptible to both, a ratio of 9 resistant to 7 susceptible was obtained in the F_2 generation. Either strain alone gave the expected 3 to 1 ratio. Later (*31*) he gave additional data including 36 F_3 families which supported his earlier interpretation. In addition he reported rootrot (caused by *Fusarium martii phaseoli* Burk.,) and mosaic resistance in beans to be partially recessive. He interpreted his F_2 results according to the ratio 9 susceptible to 7 resistant, which was supported by the performance of 183 F_3 families for rootrot and 329 F_3 families for mosaic. Parker (*39*) published a preliminary report on the crownrust of oats, *Puccinia lolii avenae* McAlpine, in which he concludes that resistance is recessive but was caused by multiple factors. Orton (*36, 37*) described the transference of wilt resistance of the stock melon to the watermelon by crossing, and similarly, resistance to wilt and rootrot was transferred from the Iron cowpea to one of the Whippoorwill type.

Among the farm crop plants that have attained commercial importance through their disease-resisting qualities, but which originated by selection or introduction, may be mentioned the following:

1. Upland and Sea Island cotton resistant to wilt. (Webber.)
2. Potatoes resistant to late blight and scab. (Jones and Stuart.)

3. Flax resistant to wilt. (Bolley.)
4. Cantaloup resistant to leafblight. (Blinn.)
5. Clover resistant to anthracnose. (Bain and Essary.)
6. Durum wheat in the Dakotas (Carleton) and Kanred wheat in Kansas (Roberts) resistant to rust.
7. Tobacco resistant to rootrot. (Johnson and Milton.)
8. Grape resistant to Phylloxera. (Viola and Ravaz.)

A genetic analysis of the resistance of these crops after crossing them with susceptible races would be required to show the type of inheritance in each case. Such investigation would be more concise and the interpretation clearer if a standard method of inoculation were adopted and a quantitative rather than a qualitative measure of resistance were used, as is being done in rust resistant work with cereals (*38*). The analysis of the inheritance of rootrot in tobacco (*21*) should prove especially interesting because of the ease with which crosses are made and also on account of the amount of genetic work already done on tobacco. It is difficult to interpret much of the past work because inoculation was left to chance or natural agencies, which allowed part of the individuals to escape infection and the resulting segregates were arbitrarily classified as resistant or susceptible, when as a matter of fact every graduation from complete susceptibility to complete immunity occurred. In some cases (*35*) different stages in the life history of the organism apparently do not have the same infective power.

Whether resistance of a given host against a given parasite will be maintained indefinitely in a given environment remains for the future to decide. Resistance in certain crops (*46*) may be only temporary. The resistance of the host might be apparently lost through the introduction of a new physiological race of the parasite to which it was susceptible. An example of such an occurrence is recorded by Levine and Stakman (*25*). They obtained a strain of stemrust from Oklahoma that could attack Kanred wheat although Kanred continued to be very resistant to the two common races of stemrust in Kansas. According to Evans (*11*) Bobs Rust Proof wheat was resistant to stemrust at Pretoria, South Africa, but was badly attacked in the Low Country of the Transvaal. This may have been due to the change in environment or to the presence of a different biologic form of the fungus encountered in the Low Country. Evans also reported an extraordinary case in which the pathogenic properties of stemrust on wheat were increased nearly five fold by passing one generation of its existence on the F_1 generation of a cross between a resistant and a susceptible variety (Bobs × Wol Koran). Bobs normally produced one-fifth as many pustules as Wol Koran, but when infected with spores from the F_1 generation it produced one-third as many. In this case the F_1 generation had apparently acted as a "bridge" to permanently increase the virulency of the rust and had also decreased the difference between the resistant and susceptible varieties. If this should prove to be a general rule, breeding for resistance would not only be without avail but would actually be a very dangerous practice on account of the danger of developing and spreading super-virulent cultures of the parasite. It is to be regretted that Evans did not grow an F_2 generation of the host to see whether super-resistant segregates could be obtained. He worked with comparatively few plants in the greenhouse, and his results may have been due to some uncontrolled factor, for other investigators have not been able to duplicate his results. Biffen (*3*) is positive that such "bridging" or increase in virulence did not occur among any of his many hybrids in England. Stakman and his associates (*45–53*) in America, after long and careful work with stemrust on its many hosts, have failed to find any indication of "bridging" or increased virulence of the parasite. They grew it on hybrids of resistant × susceptible wheat varieties from the F_1, F_2, and F_9 generations. They used different physiological races of the parasite and many species, including several genera of host material. In no case did they find an intermediary host that would increase the virulency of the rust. On the other hand, they found that the parasite became sickly when cultivated on a resistant host and often could not be maintained more than a few generations.

According to Vavilov (*58*) "bridging species" are found in *Puccinia Symphyti Bromorum* F. Müll. (M. Ward and Freeman), *Erysiphe graminis* DC. living on Bromus (E. Salmon), in *P. graminis forma* sp. *tritici* (Freeman and Johnson), and *Sphaerotheca Humuli* on Alchemilla (Steiner). It will be noted that these few cases deal with relatively weakley specialized fungi, when compared with the smuts, among which biologic races of different infecting power are known to exist; and the hosts used contain races varying from complete susceptibility to immunity. The conflicting accounts of American investigators may be explained on similar grounds. For example, Johnson (*20*) was able to transfer timothy rust *Puccinia phleipratensis* Erikss. and Henn. to barley by means of oats as a bridging host when it could not be transferred from timothy direct, but Stakman and Jensen (*47*) transferred timothy rust successfully to oats, barley, rye, wild oats, oat grass, orchard grass, wild rye, rye grass, and rough brome, without the use of a bridging host.

The biologic races of hosts and parasites, differing in resistance and virulency and the difficulty of keeping pedigreed cultures of each not only make accurate work difficult but make it almost impossible for two investigators working in different parts of the country to corroborate each other's work. "Wheat" and "stemrust" are terms not sufficiently specific for work of this kind. The particular variety of wheat and the biologic race of the rust must be known.

Plants vary also in their resistance to the attacks of insects. Torssell (*56*), in Sweden, found certain winter wheats that were not harmed by the six-spotted leafhopper while other varieties in the same field were destroyed. In 1916 in the variety test of field peas planted at the Washington Agricultural Experiment Station, the seed of Alaska peas was infested with pea weevil. At harvest time the Alaskas contained more infested than good seed, while the Bangalias growing beside them contained only a trace. The adult weevil is winged but evidently preferred the Alaskas. It is a common observation among potato growers that the Colorado potato beetle will destroy certain varieties before attacking others. For example, if Early Rose and Rural New Yorkers are planted in alternate rows in a garden, the beetles will strip the leaves of the Early Rose before harming to any appreciable extent the Rurals. But perhaps the most striking example of resistance to insect attack is that of certain grapes resistant to the plant louse *Phylloxera vitifoliae* Fitch. described by Bioletti (*4*). When resistant and susceptible varieties are crossed, resistance seems to be dominant. Such hybrids have become the foundation stock for grafting with the choice wine, table, and raisin grapes of Europe. Phylloxera feeds on the roots of the grape and produces galls or "tuberosites" which, on the susceptible varieties, enlarge enormously, and in two or three years kill the vines. This growth is caused by a poison which is injected by the insect. The resistant varieties do not react to this poison, and in addition the insect does not multiply so rapidly on it, which indicates a repellant effect of the juice of the resistant plant on the life of the Phylloxera. Between two and three million acres of vineyards in France and California have been destroyed by Phylloxera during the past 50 years and have been replanted to choice varieties that have been grafted on to resistant stocks.

Little work has been done on the inheritance of disease resistance in animals. The nearest approach to an exhaustive critical investigation in this subject, is that by Little and Tyzzer (*26*). They could transplant the carcinoma tumor designated as *J. w. A.* at will on the Japanese waltzing mouse, but the common mouse was immune to it. The F_1 generation of these two races was very susceptible, there being but 1 in 62 that failed to react. The F_2 and F_3 generations were very nearly immune, there being but 3 reacting positively out of 221. The F_1 generation, back-crossed with the susceptible parent gave mostly susceptible offspring (64 positive to 4 negative), but when back-crossed with the immune parent only immune offspring were produced (112 negative reactions). Altogether 629 mice were used in the investigation. The authors interpreted susceptibility to transplanted carcinoma tumor *J. w. A.* as being due to a complex of dominant multiple factors but considered their data insufficient to arrive at an approximation of the number involved.

From the illustrations given above it will be seen that the researches already made on the inheritance of disease resistance indicate that, in the main, whether due to physical or chemical causes, Mendelian laws of segregation and recombination control resistance phenomena generally. Artificial crossing and selection of large numbers should help clear up the difficulties in making practical use of these laws in controlling crop and animal pests.

BUNT RESISTANCE PHENOMENA IN WHEAT

THE PARASITE

There are two species of bunt, *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. *T. tritici* is the most common and is the one generally referred to in the literature, and, unless otherwise designated, is the species referred to throughout this paper.

The smuts (order Ustilaginales) were among the first recognized plagues of plants. Pliny (*41*) and Theophrastus (*54*) wrote about smut outbreaks in grains and considered them due to untoward weather and soil conditions which caused a morbid transformation, that is, putrifaction, of the tissues of the grain. In 1801 Persoon (*40*, p. 224) described stinking-smut as being due to a fungus. Meyen (*33*, p. 108–119) as late as 1841 wrote that the smut powder in a dissolved condition entered the roots and caused the sap to engender the same disease. But Kühn (*23*) was the first to prove that the fungus from the germinating spore actually enters the young wheat plant, thereby starting infection that results in the smutted head.

According to Arthur (*1*) farmers reported a bunt outbreak in 1859 in the Haw Patch district of Indiana that took half the crop. Although such epidemics are not common in Indiana, another one occurred in this same district in 1887 and in some fields caused an almost total loss.

In total harm done in reducing the world's wheat crop, bunt is second only to rust (*19*). In the wheat section of the Pacific coast in America in 1919 it is estimated that between two and three million bushels were lost on account of it. Besides the actual reduction in yield, the spores clinging to the grain and straw may be harmful to certain animals, and added expense is required to clean the grain for flour if the crop is even slightly contaminated. It is known and dreaded in all countries where wheat is grown. The countless spores in the separator during thrashing are sometimes exploded by the static electricity of the revolving cylinder and a fire is started that destroys both machinery and crop (Pl. 1, A). During thrashing the liberated spores are disseminated by the wind, and, as Heald and George (*16*) have shown, distributed at the rate of more than 35,000 spores to the square inch during a single season over distant fields about to be planted to fall wheat. Under such conditions seed treatment will not prevent a smutty crop of winter wheat the following year if the season is favorable for infection by the windborne spores. Experiments have shown that in a few weeks after the first rains, however, the spores germinate, and in the absence of wheat seedlings to infect, the fungus dies within a month or 6 weeks. Spores have been kept in the dry for 12 years which would still germinate (Woolman and Humphrey.[3])

McAlpine (*28, 29*) describes the spores of *Tilletia tritici* (Bjerk.) Wint. as globose with reticulate roughened surface 15 to 22 μ (average 16μ) in diameter. Under suitable temperature and moisture conditions the resting spores germinate in air or water. In water a long septate, unbranched mycelium is produced, whereas in air a short, septate, promycelium is produced, at the end of which are borne 3 to 10 filamentous or sickle-shaped conidia. These conidia, fusing in pairs, produce the infection threads by means of which the parasite enters the host. This is accomplished between germination and the beginning of photosynthesis of the young wheat plant. Once established, the fungus grows until fruiting time, keeping pace with the growing point without serious injury to the host. It then produces a mass of hyphae in the walls of the young ovules, which results in such a quantity of odoriferous spores as to fill the ovaries to the point of bursting—that is, making "smut balls" about the size of wheat kernels.

The resting spores are never produced except in the ovaries of the host. The parasite remains in a vegetative condition during the lifetime of the host—whether it be 2 months or 10. The physico-chemical changes that take place in the wheat plant as it approaches maturity stimulate the bunt organism to similar changes, for it quickly spreads its hyphae through the remains of the nucellus and deposits masses of its own fruiting spores in the space normally occupied by the endosperm and embryo of the wheat.

Bunt, in common with other smuts, apparently consists of but a single biologic race. Like other disease-producing organisms, it is selective for certain tissues (*24*). The nodes and growing points of the host are the tissues in which it is most frequently found in this case, and the fungus no doubt produces toxins which bring about the deformity characteristic of a smutted plant. (Pl. 1, B; 2, A.)

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[3] WOOLMAN, Horace M., and HUMPHREY, Harry B. STUDIES IN THE PHYSIOLOGY AND CONTROL OF BUNT OR STINKING SMUT OF WHEAT. U. S. Dept. Agr. Bul. (In press.)

According to Woolman,[4] bunt infection takes place during the early seedling stages, through the walls of the coleoptile. He is unable to find any trace of mycelium except in the coleoptile until the seedlings are about 8 days old. By the time they are 15 days old the mycelium has penetrated to the inner side of the second leaf sheath. As the plant grows older the mycelium penetrates farther and is carried upward mechanically with the growing point, living filaments being found most frequently at the nodes and in or near the actively growing parts. The older parts of the mycelium disappear, apparently being digested and absorbed. He finds that the so-called immune varieties are readily infected and that resistant and susceptible seedlings show no difference during the first two weeks. By the time they are a month old, however, the mycelium in the resistant varieties is more restricted in its ramifications and appears lacking in stainable cell contents. The "resistance" is evidently chemical in nature and begins to restrict the growth of the mycelium shortly after the genesis of photosynthesis. He rarely finds evidence of infection at more than one point, although he found one head containing spores of both *Tillitia tritici* and *T. levis*, which proves that two infections did take place, each functioning at maturity.

Heald (*17*) believes that multiple infection is required to develop a smutted plant. He found that Marquis wheat, which is very resistant to bunt, could endure from 500 to 1,000 spores per grain without producing bunted heads in the succeeding crop. When seed carrying 100,000 or more spores per grain were planted half the resulting crop consisted of bunted heads. Jenkins Club, which is very susceptible, produced a trace of bunt with as few as 100 spores per seed. When the inoculation reached 40,000 or more the resulting crop produced from 80 to 100 per cent of bunted heads.

If the multiple infection hypothesis be the correct explanation of this phenomenon, it might be compared to induced immunity in animals brought about by sublethal injections of fully virulent organisms. Infection in a great number of different places—that is, a lethal dose—would use up the "antitoxin" already present in the plant so rapidly that the pathogenic relationship could be established. The resistant varieties would either contain more of the "antitoxin" or a greater capacity for developing such material. Much more work will be required to establish this point. The data as published (*17*) might be interpreted as increasing the chances for infection or producing the external conditions that would make possible the penetration of the host cuticle (7). It has been shown that the amount of bunt is greatest in wheat planted for September 15 and only slightly less on October 1, at Pullman, Wash., the amount growing less and less in plantings either earlier or later. In view of this fact, as well as the fact that varieties differ in relative susceptibility, makes the proposition of determining by a spore count whether a given lot of seed needs treating, a difficult and precarious one, for winter wheat. A general prediction of the amount of smut to be expected in spring wheat would be less hazardous.

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[4] My former colleague, Mr. H. M. Woolman, has done much cytological work on the relationships of bunt and wheat, and he has kindly permitted me to make the above statements of his unpublished work.

Summarizing the evidence at hand, bunt appears to be a highly specialized parasite, consisting of but a single biologic race and existing on but a single host genus, Triticum.[5] Its spores are disseminated both on the seed of the host and by wind to fields of fall wheat. Infection takes place only during the seedling stage of the host and to be effective may require multiple infection. It produces but one crop of spores a year, these being deposited in the wheat ovaries following the flowering period. Next to rust, it is the most destructive parasite of wheat. Besides the actual loss due to bunted heads, additional losses occur through feeding smutty grain to animals, through lowering the vitality of the seed by treatment with disinfectants, through the time and expense of treating the seed, through fires caused by explosions of spores during thrashing, through extra cleaning required in milling, and through experiments of farmers, such as planting out of season, changing seed, and trying other unadapted plants in an effort to avoid a smutty crop.

THE HOST

There are eight commonly recognized species of wheat in American agricultural literature. According to Tschermak (57) Einkorn, *Triticum monococcum* is quite distinct from the others and generally will not cross with them, although Blaringhem (5) succeeded in crossing it with durum and Polish wheat. Emmer, *T. dicoccum* Schr., is thought to be the progenitor of the other six. Durum, *T. durum* Desf., Polish, *T. polonicum* L., and Poulard, *T. turgidum* L. are inter-fertile but show about 50 per cent sterility when crossed with the other three. Common wheat, *T. vulgare* Vill., Club wheat, *T. compactum* Host., and Spelt, *T. spelta* L. are all fertile inter se.

Triticum generally shows intersterility with other genera, although crosses have been made with Secale (rye), but the F_1 generation is nearly always sterile, rarely producing seed. Love and Craig (27) reviewed former work on wheat-rye hydrids and reported a cross between Dawson Golden Chaff and common rye in which they obtained a single plant in the F_1, F_2, and F_3 generation, there being but one seed produced in each of the first two generations. The F_3 generation was not so nearly sterile, for it produced a number of seeds from which a variable population of F_4 segregates were obtained.

In common with many other members of the grass family the bread wheats have hollow stems closed at the nodes and two-ranked parallel-veined leaves consisting of sheath and blade. The sheath envelopes the stem with the edges overlapping. The flowers are perfect, arranged 1 to 6 on a spikelet, and the spikelets alternate on the rachis to form a spike. Two empty glumes inclose each spikelet. Each flower is inclosed in a floral glume on the outside and a palea on the inside and consists of three stamens with slender filaments and a 1-celled ovary beneath two styles with plumose stigmas. Wheat generally is self-fertilized, but natural hybrids sometimes occur in arid climates.

After germination the plant passes through a vegetative stage in which it produces a cluster of leaves (the stool), tillering from a zone of active

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[6] Since completing this manuscript I found two smutted ryelike plants in an F_2 generation of Hybrid 128 wheat Rosen rye. The characteristic odor and reticulated surfaces of the spores make it almost certain that the casual organism was *Telletia tritici*, (Bjerk.), Wint. The same season (1921) F. J. Stevenson found two heads of what looked like bunt on common rye in our cereal nursery at Pullman, Wash., near the place where the two rye-wheat hybrid plants had been found.

tissue near the surface of the earth. With the approach of summer the stems elongate rapidly, the seeds develop, and the plant dies. The vegetative stage is completed in from one to eight months, depending upon temperature and variety.

The vegetative period exhibits some interesting variations. A fundamental varietal agronomic difference of great practical importance, which is entirely ignored in most taxonomic nomenclature, is the habit of growth during this vegetative period that distinguishes winter and spring wheats. A typical winter wheat, in the north temperate zone, will not head out or produce grain if planted in the spring. It remains as a green grass clump all summer and dies the following winter. If it is pastured or the leaves frequently cut off during the summer, it may live through the winter and the following summer head out and produce a normal crop of grain. Spring wheat, planted in the spring, passes through the vegetative stage in a few weeks and then the culms rapidly elognate, the head develops, and a normal crop is produced. If both are planted in the fall, the winter wheat lives through the freezing and thawing of a severe winter and produces a normal crop the following harvest under conditions in which the spring variety would winterkill. The time required from flowering to the maturing of the grain varies greatly with the changing climatic conditions. High temperatures, bright sunlight, and strong winds favor rapid maturation. Cool, cloudy weather with little wind favors the maximum length of time between flowering and maturity. The elongation of the stems, flowering, and fruiting are completed in from 30 to 60 days or longer, depending upon weather conditions.

Most of the cultivated wheats are of the species *Triticum vulgare*, but in certain districts the durum and club wheats predominate. Durum is produced in the Dakotas and western Minnesota principally, and the club area is confined, for the most part, to the Pacific Northwest.

Many investigators have classified comparative susceptibility to disease according to genera and species. (Jaczewski (*18*), Carleton (*8*), Freeman and Johnson (*12*), Stakman (*52*), Kirchner (*22*), and Reed (*42*).) For wheat they have shown that einkorn is generally resistant to all rusts and mildews. The durums, polish, poulard, and some of the emmers are partially resistant, but the common and club wheats, the spelts and part of the emmers are generally classified as susceptible. This shows a remarkable parallelism with the sterility groups and suggests the probability that the physical and chemical factor differences that cause sterility are also responsible for the differences in resistance. It would be expected, if this were the case, that specific sterility factors would be linked with resistance, and both would be linked with the morphological differences that make up the species. Hayes, Parker, and Kurtzweil (*15*) actually found such linkage, together with the expected sterility, in crosses of common with both durum and emmer. Linkage was not complete, however, for out of a large number they obtained a very few segregates of common type that were resistant to stemrust and some of emmer and durum type that were susceptible. From this it appears that the physico-chemical factor complex responsible for sterility in crosses of so-called immune and susceptible races is not identical with those producing resistance, although very closely associated. In fact, certain sterility factors may be the identical factors that cause certain kinds of resistance, for resistance is specific for each parasite. A wheat may be susceptible to stemrust and resistant to yellow rust, or resistant to mildew and susceptible to both rusts (*2*, *9*).

Bunt, like the rusts, finds certain species more congenial hosts than others. Table I, compiled from the tests of 1919 and 1920 at the Washington Agricultural Experiment Station, Pullman, Wash., shows the comparative resistance of the eight species as tested.

TABLE 1.—*Comparative bunt resistance of the eight species of wheat*

Species.	Number of tests.[a]	Total number of plants counted.	Percentage of bunt produced.
Einkorn (*T. monococcum*)	4	87	0.0
Polish (*T. polonicum*)	3	66	7.7
Emmer (*T. dicoccum*)	10	155	9.6
Spelt (*T. spelta*)	21	515	9.7
Durum (*T. durum*)	40	1,256	29.3
Poulard (*T. turgidum*)	9	264	33.5
Club (*T. compactum*)	98	4,296	64.1
Common (*T. vulgare*)	666	25,009	70.2

[a] The actual number of distinct races or sorts was but slightly over 500 in round numbers. Many of the 1919 plantings were duplicated in 1920.

Taking the average percentage of bunt produced as the index of resistance, the eight species may be arranged into four groups. Einkorn is immune, polish, emmer, and spelt are very resistant, durum and poulard are intermediate, and club and common are very susceptible. It would require a much more exhaustive test to establish these findings as general laws for specific bunt resistance, but there are two deductions that may be made. 1. Species of Triticum differ in their susceptibility to bunt and in a manner analogous to specific rust resistance and also analogous to their genetic relationships. 2. Spelt forms a notable exception, falling into the very resistant class instead of the susceptible class that its genetic and rust-resistance relationships call for. These relationships are established by the work of several investigators (*15*, *57*, *58*), and the different behavior of spelt in respect to bunt resistance is established in Table 1 by the performance of 21 rows and more than 500 plants.

It has been found that the common and club wheats vary within rather wide limits among the different races of the same species, a few selections of both being very resistant, more being intermediate, but the majority being very susceptible.

Resistance to some diseases seems to break down when the host is grown in another environment. The information available on bunt resistance indicates that a wheat resistant to bunt will remain so in all climates. For example, Turkey (Washington, No. 326) is resistant under conditions favoring maximum infection at the experiment stations of Kansas, Minnesota, California, Oregon, Idaho, and Washington.

A comparison of a large number of varieties, known to be pure lines, in two districts of distinctly different climate should give a clear idea of the probable constancy of bunt resistance in different environments. This can best be done by means of a correlation table in which the comparative resistance of each variety in one locality is measured in relation to its comparative resistance in the other locality. If there is perfect agreement, the coefficient of correlation is 1. If they vary independently in each locality, without regard to the comparative resistance in the other, the coefficient of correlation equals 0. Table II presents such a correlation.

TABLE II.—*Correlation of 150 varieties of wheat in respect to bunt resistance* [1]

Class centers.	5	15	25	35	45	55	65	75	85	95	Pullman frequencies.
5	4	....	1	....	1	....	....	....	....	....	6
15	1	1	....	1	....	....	....	....	....	....	3
25	1	....	2	....	....	....	1	....	2	....	6
35	....	1	....	....	2	....	....	3	....	....	6
45	....	....	2	3	....	1	2	1	1	....	10
55	....	1	....	....	1	2	1	1	....	2	8
65	....	....	1	2	2	5	2	2	4	4	22
75	....	1	....	2	1	2	....	4	5	1	16
85	1	....	1	....	1	1	3	4	6	5	22
95	....	....	....	1	1	....	1	6	12	30	51
Moro frequencies	7	4	7	9	9	11	10	21	30	42	150

[1] Percentage of bunt at Moro, subject; percentage of bunt at Pullman, relative; 1919.
Coefficient of correlation=0.654±0.0315.

A comparison of 150 selections of wheat (mostly *Triticum vulgare*, a few being club and durum) tested at Pullman, Wash., and Moro, Oreg., show a correlation coefficient, according to Table II, of 0.654 ± 0.0315. Moro has an arid climate with an average rainfall of 11.6 inches. The elevation is 1,800 feet; the soil, a fine silt loam. Pullman has an annual rainfall of 21 inches, a clay loam soil, an elevation of 2,500 feet, and a lower summer temperature than Moro. The Moro data were obtained by counting the heads of bunt and wheat from which the percentages were figured. The Pullman figures were obtained by the combination plant and head count described on page 460. Taking into account the differences in climate and methods, the high correlation is significant. It shows that resistance and susceptibility are fundamental differences and are not easily changed by environmental conditions. A large number of these varieties have been tested at Davis, Calif., Aberdeen, Idaho, and Corvallis, Oreg., under wide differences of soil and climate, and the indications are that the common varieties that are decidedly resistant or susceptible are outstanding in those respects at all places where tested. Table II shows only four varieties less than 10 per cent bunt at both Stations, but 30 varieties more than 90 per cent susceptible. The average of all varieties at both Stations is 70 per cent, which is very near the figure given for the *T. vulgare* group in Table I, in which a much larger number of varieties is represented, covering the two-year test (1919 and 1920) at Pullman.

It is very seldom that a row of wheat, containing, say, from 50 to 100 plants produces nothing but bunt heads, no matter how susceptible the variety is or how favorable the conditions for infection are. A small number of wheat heads, and usually a few plants, escape the fungus and produce normal seed. It might be assumed that these occasional plants were resistant mutants. To test this possibility 10 bunt-free plants of hybrid 143 were selected in 1913, and for three years smut-free plants from the most resistant row were selected. At the end of the third year the selected plants produced 85 per cent of bunt as compared with 80 per cent for the unselected check. Other tests of selections from Turkey and Red Russian add proof to the conclusion that the bunt-free plants in varieties planted under conditions favoring maximum infection are not resistant mutants but rather escape infection by accident.

BREEDING FOR BUNT RESISTANCE

METHODS

In testing different wheats for comparative resistance to bunt, conditions favoring maximum infection were, as nearly as possible, maintained. The seed was inoculated with fresh viable spores just before planting. This was accomplished by stirring into each packet of seeds from 1 to 5 per cent of its weight in bunt spores, so that each seed was literally blackened by spores clinging to it, besides a surplus of loose spores among the seeds. The inoculum was obtained from the smutted heads of many different varieties of wheat in the field, in order to get a representative sample of the organism in its native environment. The heads were ground up and the spores sifted out and kept in a cool, dry place during the planting season. The packets of inoculated seeds were planted by hand in rows 18 inches apart. The seeds were spaced 4 to 6 inches apart in the row to avoid confusion in separating the plants at harvest time. Each experiment, whether a variety test or an F_3 family, was planted at approximately the same date to avoid error due to changing seasonal conditions. As many as 10 men were required at times in order to plant a given experiment in a single day.

The field was kept free of weeds and volunteer grain by rotation and cultivation, corn and field peas preceding the cereal nursery in a 3-year rotation in every case. The rainless summers of eastern Washington make clean cultivation under these conditions comparatively easy. A stake bearing a printed label with date, name of experiment, name of variety and pedigree number was placed at the end of each row. The records at harvest time were obtained as follows: To get a quantitative measure of resistance, the plants of each row were pulled and separated into three piles (bunt-free, all bunt, and part bunt); and the number of plants in each was recorded in a field note book. The partly bunted plants were then divided into heads of wheat and heads of bunt, and the numbers were entered in their proper columns. With these five figures it is easy to reduce the amount of bunt in a given row to terms of a single number for direct comparison with the amount produced by any other row. The computation used gives the total bunt in terms of percentage of the whole row, according to the formula $ab+c=d$, in which a is the percentage of bunted heads on the partly bunted plants, b is the percentage of partly bunted plants in the row, c is the percentage of entirely bunted plants in the row, and d is the total percentage of the row that is bunted. The computations were made with a calculating machine and checked with a slide rule. The results thus obtained are not materially different from those obtainable by a straight head count of all the heads in the row. This method takes much less time and gives information that a straight head count would not give—that is, the quantity of partly bunted plants and the amount of wheat produced on them, which in itself is an important measure of resistance, and is useful in checking errors in counting or computations.

The work was all carried on out of doors, in the field, the main care being to have uniform conditions for all the rows of a given test, so that the differences in bunt resistance would be attributable directly to differences in the constitutional make-up of the wheats compared.

PARENT STOCK

Of the eight varieties to be discussed in relation to their bunt resistance, six are of wide commercial importance. Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones Winter Fife are the five most important winter wheats in the State of Washington and are named in the order of their importance from the standpoint of total production. They represent more than three-fourths of the winter wheat produced in the Northwest. Alaska is not commercially grown except in isolated sections where an occasional farmer has been the victim of propagandists who have made extravagant claims for it as a wonderfully productive and hardy wheat. Of the two spring varieties, Marquis is third in production in Washington and is universally grown in the Great Plains area of the United States and in northwestern Canada. Florence is of some importance in Australia but has not been grown in America except for experimental purposes.

Taxonomically all are *Triticum vulgare* except Hybrid 128, which belongs to *T. compactum* and Alaska which is a poulard, *T. turgidum*. Turkey, Florence, and Alaska are resistant (Pl. 2, B) and Hybrid 128 and Jones Winter Fife are susceptible to bunt under all conditions. Fortyfold and Red Russian are intermediate but approaching the susceptible varieties (Pl. 3, A). Marquis is resistant when sown in the spring but intermediate or susceptible when fall-sown. Table III gives the amount of bunt produced in the tests from 1915 to 1920. The season of 1917 was very unfavorable, and the wheat did not come up till spring. Since none of the varieties produced any appreciable amount of bunt, the record of that year is omitted.

TABLE III.—*Annual percentage of bunt produced on parent stock for the years 1915 to 1920, with the 3-year average for 1918 to 1920, and the total number of plants in the tests during the latter period*

Variety.	Percentage of bunt.						Total number of plants in 3 years 1918–1920.
	1915	1916	1918	1919	1920	Average 1918–1920.	
Turkey	4.6	1.7	0.6	2.4	7.6	3.5	2,883
Marquis [a]	....	....	3.4	7.3	3.5	4.7	813
Florence	....	....	.9	8.1	12.2	7.1	880
Alaska	....	....	.0	7.1	20.1	9.1	312
Red Russian	67.8	85.6	49.9	77.4	56.1	61.1	2,118
Fortyfold	73.5	71.2	49.6	76.4	65.2	63.7	479
Hybrid 128	86.6	96.4	63.0	98.1	77.6	79.6	685
Jones Winter Fife	89.3	87.4	78.3	94.2	73.6	82.0	317
Average first 4 varieties	....	....	1.2	6.2	10.8	4.5	....
Average last 4 varieties	79.3	85.2	60.2	86.5	68.1	75.9	....
Average of both resistant and susceptible [b]	....	....	30.7	46.4	39.5	38.9	....

[a] Marquis was spring-sown. All the others were sown in the fall throughout the tests.

[b] See Plates 2, B, and 3, A, for the type of heads of these eight parent varieties.

There is considerable variation from year to year, but the varieties generally maintain their relative positions. The four resistant ones are always comparatively resistant and the susceptible ones are always comparatively susceptible, regardless of seasonal fluctuations. Turkey, representing the extreme resistant type, and Fife, representing the extreme susceptible type, show less variation than the others. Marquis shows less variation than any of the others during the three years recorded and is so uniformly resistant that farmers often take advantage of it and do not treat the seed before planting. It was a matter of considerable surprise, therefore, when it was discovered that Marquis was susceptible when planted in the fall. In 1919 a fall sown row of inoculated seed produced 661 heads, 489, or 74 per cent, of which were bunted. The same phenomenon was observed in 1920, the 14 plants that survived the winter producing 31 per cent bunt, compared with 3.5 per cent produced from a spring planting of 90 plants in the same field. The record of Florence, the other spring wheat, is taken from fall sowings but is somewhat more resistant when spring-sown. Evidently the resistance of Marquis is different from that of the others, inasmuch as it is neutralized by the lowered temperature or the winter rest period. There seems to be no peculiar habit of growth or taxonomic difference associated with this loss in resistance, but it is a constantly recurrent phenomenon obtaining wherever tested. An example of the uniformity of these reactions is shown in the records from Davis, Calif. Seed of the three varieties Turkey, Florence, and Marquis was sent to that Station to be tested for bunt resistance in the season of 1920. Plantings of each were made November 4 and December 21, 1919. More than a thousand heads of each were produced in 1920, of which Marquis contained 37 per cent of bunt heads, Turkey and Florence containing but 7 and 6 per cent, respectively. The spring sowing of Marquis was uniformly resistant.

This does not mean that all the others have the same kind of resistance. In fact they have not, according to the segregation tests of their hybrid progeny, as will be shown later. It seems probable, from a study of Table III, that the different wheats do not react the same to climatic influences from year to year. For example, in 1920 the resistant varieties (with the exception of Marquis) produced more than twice as much bunt as their 3-year average shows, while the four susceptible varieties produced an average of 7.8 per cent less in a similar comparison. The reverse condition obtained in 1916, while in 1918 all varieties produced less than normal, and in 1919 all but Turkey and Alaska produced more than normal. It is very evident that a "good" or "bad" smut year does not apply equally to all varieties.

The percentage of bunt produced on the partly bunted plants was much less on the resistant than on the susceptible varieties, the ratio being 25 to 67 when the four resistant varieties of Table III are compared with the four susceptible ones. This may be taken as an argument in favor of the physico-chemical nature of resistance, for here there can be no question about the plants being infected. The difference is plainly one of greater incompatibility of the resistant hosts. The ratio of total bunt 4.5 to 75.9, however, shows a much stronger contrast and is the just and proper one to use as a quantitative measure of resistance or susceptibility.

SEGREGATION OF HYBRIDS

Only a few of the possible combinations of the eight parent types have been made, but the data obtained are sufficient to show the general type of the inheritance to be expected, and the crosses described below have been tested in sufficient quantities and over a long enough period of time to warrant the conclusion that resistance is definitely heritable according to the commonly recognized laws of genetics. Three types of crosses have been made: resistant × resistant, resistant × susceptible, and susceptible × susceptible. Because a smutted plant is automatically eliminated from further testing, as it produces no progeny, the seed for the F_1 and F_2 generations were not inoculated with spores but were treated with formaldehyde instead, so that both susceptible and resistant segregates were saved for the F_3 generation, which was thus the real beginning of the test. F_1 sibs had been saved, however, so that F_2 sib rows could be tested with the F_3's the same season, under the same conditions. Thus the parent varieties, the F_2's and the F_3's were all tested at the same time in the five crosses that have been carried on in sufficient numbers, and under such conditions as to make the results significant. The amount of bunt produced per row in the segregates of the F_3 generations has been arbitrarily put into 10 groups, each class having an amplitude of 10 per cent. The numbers in each class of 10 crosses is given in Table IV.

TABLE IV.—*Number of F_3 rows falling into each class, when the percentage of bunt produced from 0 to 100 is divided into 10 equal parts*

Variety.[a]	Number of rows falling into class with average percentage of bunt—										Total number of rows.
	5	15	25	35	45	55	65	75	85	95	
A × T	6	1	0	3	0	0	1	1	1	1	14
T × F	101	19	18	12	6	6	3	2	1	0	168
T × H	15	13	9	23	28	33	36	25	11	1	194
T × R	17	9	6	1	4	2	1	2	0	0	42
Fd × T	41	64	37	63	97	104	85	59	38	5	[b] 593
A × J	6	0	0	2	2	0	1	4	9	0	24
Fd × R	1	17	34	55	40	27	5	2	0	0	181
H × M	0	0	0	0	0	0	0	5	53	276	334
A × R	1	0	2	1	0	1	1	2	1	2	11
A × Fd	1	0	1	3	2	1	6	5	1	1	21

[a] T=Turkey, A=Alaska, F=Florence, H=Hybrid 128, Fd=Fortyfold, J=Jones Winter Fife, M=Marquis, R=Red Russian.

[b] From 42 plants selected at random from an F_3 population, which makes these figures really F_4's in which there has been no selective elimination of susceptible segregates in earlier generations.

The average number of plants per row varied in the different crosses from 12.6 to 60.8, and more than 60,000 plants were counted in determining the bunt percentages of the 1,582 rows listed in Table IV. The fact that the tests were made during four different years makes comparison difficult, in one way; yet it tends to add weight to the general conclusions regarding the inheritance of resistance, because of the very fact that the different seasons are included. All of the rows in which Alaska was one of the parents were grown in 1916. The Turkey crosses (except with Alaska and Fortyfold) were tested in 1918. Hybrid 128 × Marquis was tested in 1919, and the other two, Fortyfold × Turkey and

Fortyfold × Red Russian, were tested in 1920. Only the five crosses in which the number of rows exceed 100 will be considered in detail, as the others are too few in number for analysis. The others may be discussed briefly as follows: All four of the crosses in which Alaska was the original male progenitor produced a high degree of sterility, which, combined with lack of winter hardiness, made it difficult to obtain large numbers. The figures as given, however, show that there is segregation, as both resistant and susceptible sorts were obtained in every case. Moreover, the susceptible classes occur with greater frequency than the resistant ones in the crosses of Alaska and the three susceptible varieties, Jones Winter Fife, Fortyfold, and Red Russian. Crossed with the resistant Turkey, a majority of the F_3 rows are resistant. In fact one of the selections which was sent to the Oregon Station has been found more resistant than either parent there and has proved so promising from a commercial standpoint that it has been continued to the seventh generation. Five F_4 selections and 18 selections in the F_5 generation were grown at Pullman from one F_3 row that was entirely bunt-free, and never a trace of bunt could be found in any later generation, although Turkey

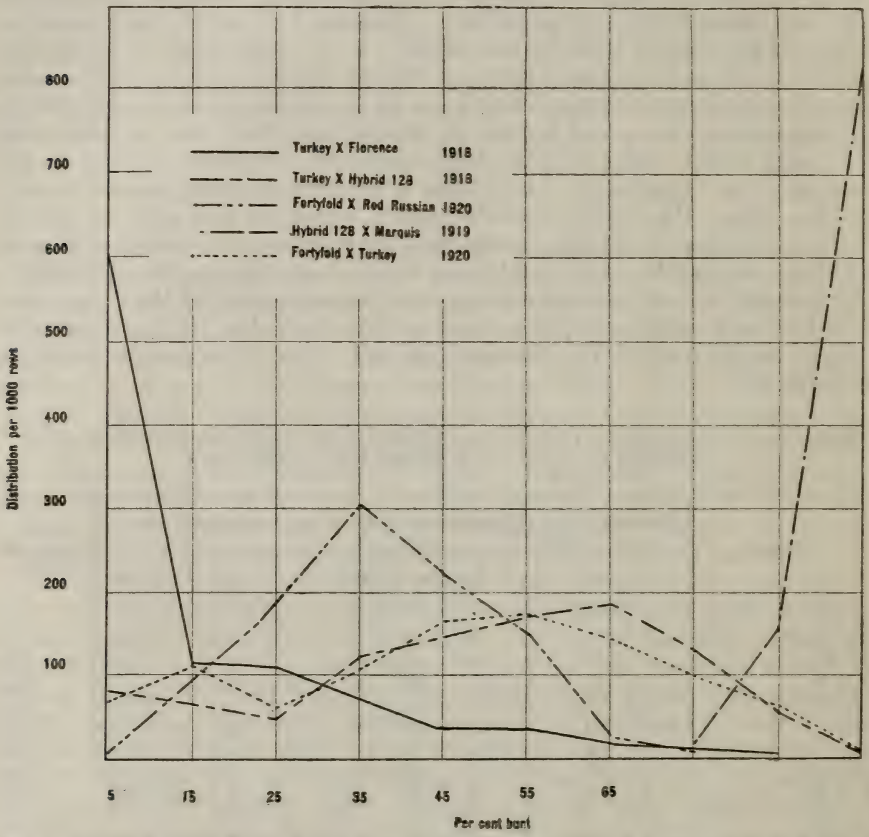

FIG. 1.—Graphical representation of bunt resistance in the F_3 generation of 5 different types of crosses. Three classes of parents were used: Resistant, Turkey (3.5), Florence (7.1); Intermediate, Fortyfold (63.7), Red Russian (61.1); Susceptible, Marquis (74). Hybrid 128 (79.6). Figures in parentheses refer to average percentage of bunt produced under conditions favoring maximum infection.) The crosses of Hybrid 128 with Turkey and Marquis reproduce only the extremes of the parents. In the other crosses transgressive inheritance occurred—that is, segregates were obtained more resistant and more susceptible than the extremes of the parents.

and Alaska, the parent strains, produced a small amount of bunt each year.

An exhaustive test of Turkey × Red Russian was attempted in 1917, but the season was so unfavorable that the grain winter-killed badly and much of it did not germinate till spring, during which time the bunt spores with which the seeds had been inoculated probably perished, for out of 513 rows planted, but 2 produced as much as 10 per cent of bunted heads. Random selection of 42 plants from this F_3 generation gave a preponderance of resistant type in the F_4 generation but was not carried further.

There are five families in which the numbers are sufficiently large and the seasons sufficiently favorable to give results that are dependable for purposes of comparison. The distribution of the different classes has been refigured on the basis of the number of each class per 1,000 rows and the result put in the form of curves in figure 1 in which the ordinates represent the number in each class and the abscissas represent the different classes. There are four distinct types of curves, one in which the 5 per cent class predominates, one in which the 95 per cent class predominates, one in which the 35 per cent class predominates, and two in which the major predominating class is 55 or 65 per cent and a minor class predominating on the resistant end of the curve, and a low center at the 25 per cent class. These two similar crosses are not so similar in reality as they appear from the position of the curves, for the data from which the curve representing the F_3 generation of Turkey × Hybrid 128 was taken was obtained in 1918, while the other curve representing the data of the F_3 generation of Fortyfold × Turkey was obtained in 1920. Reference to Table III shows that the three parent wheats produced an average of 13 per cent more bunt in 1920 than in 1918. If the curves were corrected for this seasonal difference, the F_3 generation of Turkey × Fortyfold would show a parallel curve that would be from 10 to 20 per cent more resistant than the F_3 generation of Hybrid 128 × Turkey. Turkey is one of the parents in both crosses, so the difference in the two curves must be due to the difference in the inheritance of the other two parents, Fortyfold and Hybrid 128. It seems more than coincidence that the 5-year average of the two wheats (Table III) should show 17 per cent less bunt produced by Fortyfold than by Hybrid 128. In fact the F_3 generation of Fortyfold × Turkey shows a mean of 2.3 per cent less bunt than the mean of the other F_3 generation nothwithstanding the more favorable season for bunt in which it was grown. Any combination of the parents in question shows from 10 to 15 per cent more bunt in 1920 than in 1918. The seasonal differences of each parent is, for Turkey, 7 per cent, for Hybrid 128, 14,6 per cent, and for Fortyfold, 15.6 per cent. It seems, therefore, that the curves in question should be separated, on account of seasonal differences, by at least one class, or somewhere between 7 and 15.6 per cent. In other words if the F_3 generation of Turkey × Hybrid 128 had been tested in 1920 instead of 1918 the 194 rows tested would have produced between 7 and 14 per cent more bunt on the average than they did. This would have been between 9.3 and 16.9 per cent more than the F_3 generation of Fortyfold × Turkey actually did produce in 1920. The picture of the inheritance of these two crosses is one in which susceptibility is dominant, with the susceptible segregates fluctuating around the mean of the susceptible parent in each case. A study of the material in the field revealed an important difference in the two F_3's that the figures in Table IV or the curves in figure 1 do not show clearly, namely, that transgressive segregation occurred in the F_3 generation of Fortyfold × Turkey, there being five rows decidedly more resistant than Turkey and many rows more susceptible than Fortyfold. This phenomenon was not observed in the other F_3 generation, in 1918. Turkey was just as resistant and Hybrid 128 just as susceptible as the extremes of the segregates. This suggests the idea that Fortyfold carries an element of resistance different than the elements of resistance in Turkey, which, added to it, produces a wheat more resistant than Turkey, and without which the segregate becomes as susceptible as Hybrid 128. In susceptible segregates this Fortyfold element of resistance has a value of from 10 to 20 per cent, but in resistant segregates the value lies between 1 and 7 per cent, depending upon the season. It will require tests in later generations to establish this hypothesis beyond question, and this is being done.

It is difficult to place these phenomena of inheritance on a factorial basis on account of the seasonal fluctuations and the quantitative and comparative nature of the material. It is quite evident, nevertheless, that Turkey has several times as much resistance to bunt as Fortyfold. It is also evident, from the performance of the segregates of Turkey × Hybrid 128 in the F_4 and F_5 generations, that this "Turkey resistance" splits up into its component parts when crossed with other wheats of different constitution. Table V shows the comparative resistance of 144 F_4 rows (the offspring of 144 F_3 plants, selected from nine different F_3 rows), in comparison with the parent rows from which they came.

The nine F_3 rows from which the selections were made were the most resistant segregates of beardless, club, winter hardy type (characters which were also being studied). It has been shown in an earlier paper (*14*) that resistance in this cross is not linked with the external characters studied. In comparing the third column, "Bunt in F_3 generation" with the others, it must be borne in mind that these figures were obtained in 1918 and that the F_4 percentages were obtained in 1919, in which year (according to Table III) the parents showed 18.4 per cent more bunt than in the year in which the F_3 generation were tested.

TABLE V.—*Distribution of bunt resistance of Turkey × Hybrid 128 in the F_4 generation from 9 resistant F_3 rows*

F_3 row No.	Number of plants selected from each row.	Average number of F_4 plants per row.	Bunt in F_3 generation.	Average bunt of F_4 rows.	Range of bunt in F_4 rows.	
					Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1387	26	71	1.9	12.7	5.6	35.0
1304	22	56	4.8	18.7	6.5	39.6
1310	2	50	6.4	32.0	25.9	38.1
1353	14	66	6.6	13.6	5.6	25.4
1312	29	63	7.0	13.2	4.3	26.7
1284	12	70	7.1	25.3	12.7	40.3
1297	14	42	9.8	33.2	11.6	59.5
1357	8	69	13.4	26.6	16.6	41.7
1299	17	46	14.6	34.8	15.5	54.7
Average	16	59.2	8.0	23.3	11.6	40.1

The season of 1919 was very favorable, and an average of 60 plants per row was obtained. Four of the 9 F_3 segregates produced F_4 progeny almost as resistant as the resistant parent, Turkey. The range of bunt produced by the different F_4 rows indicates that the F_3 row from which they were selected was probably heterozygous for resistance in every case, the range being from 12.2 to 39.2 per cent, which is a larger fluctuation than Turkey. (Ten rows of Turkey were grown in this same series, for comparison, the extremes of variation being 0.7 and 6.9 per cent.) There was, however, not one of the 144 rows in which the plants did not evidence some of the resistance of the Turkey parent, for the susceptible parent, Hybrid 128, growing alongside of the F_4 rows contained 98 per cent of bunt, whereas the worst F_4 row produced less than 60 per cent. The difference was even more outstanding in the field than the figures indicate. Taxonomically the F_4 rows resembled very closely the susceptible Hybrid 128, but the bunt-infected plants were very different. Once a plant of Hybrid 128 became infected it seldom produced any wheat. Out of 125 infected plants but 3 produced wheat, while 25 of the F_4 rows produced some heads of wheat on every one of the infected plants, and only 11 of the 144 F_4 rows contained more than 10 wholly smutted plants.

The plants in 5 of the 29 F_4 rows from F_3 row No. 1312 were superior in yield and quality and were very resistant; they became the progenitors, therefore, of the F_5 selections, five F_4 plants being selected from each row. Five plants were also selected from each of two other F_4 rows for test in the fifth generation. Thus the individuals of 35 F_5 rows representing 3 F_3, and through them 7 F_4 families were tested for bunt resistance in 1920. Ten of these rows—that is, 2 of the F_4 families—were selected for susceptibility instead of resistance to see whether the amount of bunt could be increased by selection after two generations of selection in the opposite direction. The data are summarized in Table VI.

TABLE VI.—*Distribution of bunt resistance in the F_5 generation of Turkey × Hybrid 128 selected from five resistant and two intermediate F_4 families*

F_4 row No.	From F_3 family row No.	Average number of F_5 plants per row.	Bunt in F_4 row.	Average bunt of 5 F_5 rows.	Range of bunt in F_5 rows.	
					Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1434	1312	42	4.3	13.3	3.3	22.2
1470	1387	42	7.5	19.4	16.1	22.9
1425	1312	52	7.9	9.1	3.1	15.2
1441	1312	59	8.2	18.3	9.2	35.8
1445	1312	48	8.5	14.7	9.1	18.8
1426	1312	60	27.7	23.2	10.8	37.8
1392	1299	45	37.5	48.3	28.8	67.7

The five plants selected from row 1470 were apparently homozygous for resistance in the F_4 generation but lacked an element of resistance that Turkey possesses, for they varied around the mean 19.4 per cent while Turkey in 1920 varied around the mean 7.6 per cent—the worst year for Turkey since the tests began in 1914. The numbers 1426 and 1392 of Table VI show that they were heterozygous and that susceptibility can be increased by selecting in that direction, for one row was within 10 per cent of being as bad as Hybrid 128.

The total populations of the F_4 and F_5 generations are presented in figure 2 in terms of the distribution of rows in the different classes per thousand, similar to the F_3 distribution in figure 1. It will be seen that the F_4 and F_5 generations very nearly parallel each other. If the two susceptible lines had been eliminated, the other five would show decidedly greater resistance than the F_4 generation in spite of the fact that resistant varieties generally were twice as badly smutted in 1920. (See Table III.) Figure 2 may be taken to fairly represent the first peak of resistant segregates of the F_3 curve in Figure 1. No doubt subsequent selections will isolate the full resistant qualities of Turkey in the progeny in the homozygous condition. It is not unlikely that some of the F_5 rows are already in that condition and can not be changed by selection.

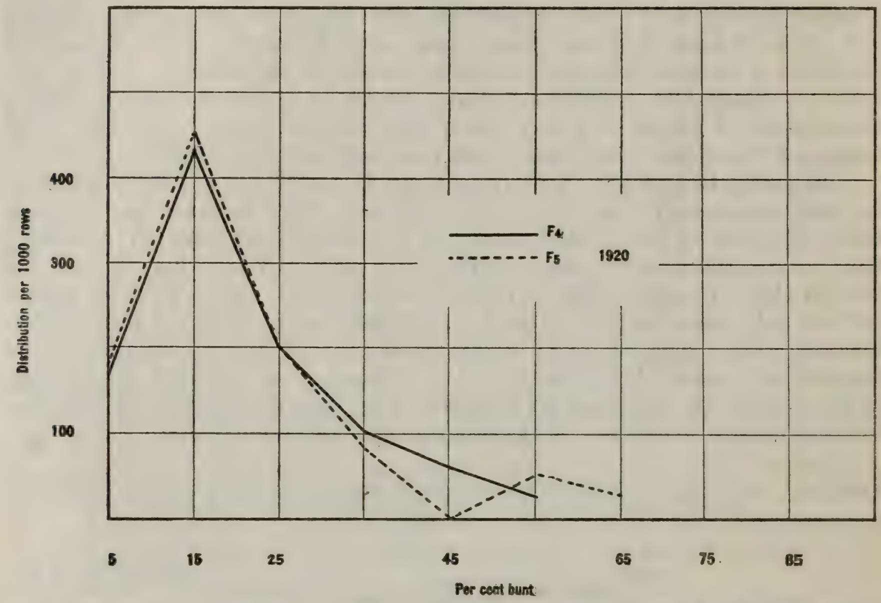

FIG. 2.—Graphical representation of resistant segregates in later generations. The similarity of the F_4 and F_5 segregates indicates the constancy of the selections. The increase on the susceptible end of the F_5 curve is due to the selection of 10 families in that direction from the F_4.

For convenience in summarizing the data in respect to the bunt resistance of Turkey and Hybrid 128 and the progeny of a cross between them, Table VII has been prepared. It should be kept in mind that the result of a given generation represents the constitution of the generation preceding. Thus the results of the F_3 generation show that the F_2 generation reproduced the extremes of the parents with a rather indefinite frequency center near each parent center. The great majority tended toward the center of Hubrid 128, indicating dominance of susceptibility. Similarly the F_4 and F_5 class frequencies indicate the constitutional resistance of the F_3 and F_4 generations. Each later generation shows less variation than the one preceding, as would be expected in a self-fertilized race when selected in the direction of the recessive class center.

TABLE VII.—*Frequency distribution for resistance to bunt in a cross between two varieties of wheat, one resistant, the other susceptible*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Turkey......	1918	0.5	7	....	....	....	....	....	....	....	....	....
	1919	.6	11	....	....	....	....	....	....	....	....	....
	1920	2.4	5	....	....	....	....	....	....	....	....	....
Hybrid 128...	1918	Not tested.	....	....	....	....	1	5	....	2	....	....
	1919	63.0	....	....	....	....	....	....	....	....	....	2
	1920	98.1	....	....	....	....	....	....	1	3	3	....
T×H F_2....	1918	Not tested.	....	....	....	1	....	1	....	1	....	....
T×H F_3....	1918	.....do.......	15	13	9	23	28	33	36	25	11	1
T×H F_4....	1919	1.9	12	10	3	1	....	....	....	....	....	....
		4.8	1	12	6	3	....	....	....	....	....	....
		6.4	....	....	1	1	....	....	....	....	....	....
		6.6	3	10	1	....	....	....	....	....	....	....
		7.0	8	16	3	....	....	....	....	....	....	....
		7.1	....	5	3	3	1	....	....	....	....	....
		9.8	....	2	5	2	3	2	....	....	....	....
		13.4	....	4	1	2	1	....	....	....	....	....
		14.6	....	2	6	3	4	2	....	....	....	....
T×H F_5....	1920	4.3	1	3	1	....	....	....	....	....	....	....
		7.5	....	3	2	....	....	....	....	....	....	....
		7.9	3	2	....	....	....	....	....	....	....	....
		8.2	1	3	1	....	....	....	....	....	....	....
		27.7	....	2	2	1	....	....	....	....	....	....
		37.5	....	....	1	1	....	2	1	....	....	....

Table VIII gives the class frequencies of Turkey and Florence in respect to bunt resistance as well as those of the progeny obtained from a cross between them. An extra column, not given in Table VII, has been added to show the rows that were entirely without visible infection when harvested.

It will be seen that the F_2 generation (the constitution of which is shown by the F_3 class frequencies) shows transgressive inheritance, with the greatest frequency coinciding with the average resistance of the parents, if it be assumed that the 72 immune segregates varied in the intensity of their resistance in proportion to those in the direction of the susceptible end of the table. Such an assumption seems warrantable from the records of the F_4 and F_5 generations, for they were more resistant than the parents, and only a trace of bunt appeared in each generation in a few of the rows. The F_5 generation shows that 19 out of 25 rows were bunt-free (immune), and their ancestry had been bunt-free since the cross was made. Thus, for three generations these selections have not produced a trace of bunt, although planted each year under conditions favoring maximum infection—that is, the seed was blackened with spores. The parents produced an average of 3.5 and 7.1 per cent under the same conditions, and other varieities, classed as susceptible produced as much as 80 per cent of bunt.

TABLE VIII.—*Frequency distribution for resistance to bunt in a cross between two varieties of Triticum vulgare, both of which are resistant*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—											
			Immune.	5	15	25	35	45	55	65	75	85	95
		Per cent.												
Turkey 326	1918	0.5	1	6	...	...	...	...	...	...	...	...	...
	1919	.6	...	11	1	...	...	...	...	...	...	...	...
	1920	2.4	...	5	...	...	...	...	...	...	...	...	...
Florence 634	1918	.0	1	2	...	...	...	...	...	...	...	...	...
	1919	.9	...	2	...	...	...	...	...	...	...	...	...
	1920	8.1	...	1	...	2	...	...	...	...	...	...	...
326×634 F_2	1918	Not tested.	...	1	1	...	...	...	...	...	...	...	...
326×634 F_3	1918	do.	72	29	19	18	12	6	6	3	2	1	...
326×634 F_4	1919	.0	11	1	...	...	...	...	...	...	...	...	...
		.0	9	2	...	...	...	...	...	...	...	...	...
		3.2	3	8	...	...	...	...	...	...	...	...	...
		[a] 67.7	3	...	...	...	...	...	...	...	...	...	...
		.0	2	5	...	...	...	...	...	...	...	...	...
326×634 F_5	1920	.0	3	2	...	...	...	...	...	...	...	...	...
		.0	5	...	...	...	...	...	...	...	...	...	...
		.0	4	1	...	...	...	...	...	...	...	...	...
		.0	5	...	...	...	...	...	...	...	...	...	...
		.0	2	3	...	...	...	...	...	...	...	...	...

[a] This is evidently a mistake. The row next to it was immune, and it seems probable that the numbers got mixed at harvest time, for the three selections showed no trace of bunt the following year.

In 1919 an F_3 family of Hybrid 128 × Marquis was produced to test the inheritance of the resistance of Marquis to bunt. Information regarding the inheritance of Hybrid 128 had been obtained the year before from the F_3 generation of the cross between this variety and Turkey. The question arose as to whether the seed should be planted in the fall or spring, one parent being a winter wheat the other a true spring variety. If fall-sown, much winterkilling would be expected, for Marquis is not very winter-hardy. If spring-sown the true winter segregates would not produce heads and two-thirds of the others would be heterozygous for the winter factor and would produce some winter plants and some that would develop late so as to interfere with harvesting operations. The former course was decided upon and the planting was done in October, 1918. The winter was unusually mild, and a good stand was harvested the following summer. Every one was surprised to find all of the F_3 rows (334 in number) very badly bunted. The best row in the lot was three-fourths smutted, and 276 of the rows produced more than 90 per cent of bunt. The whole block of rows appeared as a mass of stinking smut which gave off an offensive odor that penetrated to windward for half a mile. An examination of some demonstration plots in another field showed that the fall-sown Marquis was very smutty. This gave a clue as to why all of the F_3 rows of Hybrid 128 × Marquis were badly bunted. The inherent resistance of Marquis was broken down by the winter rest period.

One plant each from nine of the best rows—that is, those having the least amount of bunt—and one from each of the four worst rows were selected for testing in the F_4 generation. The complete results of the parents, the F_2, F_3, and these F_4 selections are summarized in Table IX.

TABLE IX.—*Frequency distribution of resistance to bunt in a cross between Hybrid 128 and Marquis, both of which are susceptible when fall-sown*

Variety.	Year tested.	Bunt in parent row.	Number falling into class with average percentage of bunt of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Hybrid 128	1919	63.0	....	....	....	....	....	....	....	....	....	2
	1920	98.1	....	....	....	....	....	....	1	3	3	....
Marquis	1919	Not tested.	....	....	....	....	....	....	....	1	....	....
	1920	74.0	....	....	....	1	[a] 3	....	[a] 1	....	....	....
H × M F_2	1920	Not tested.	....	....	....	....	....	1	....	....	....	....
H × M F_3	1919	do	....	....	....	....	....	....	....	5	53	276
H × M F_4	1920	75.9	....	....	....	....	....	1	....	....	....	....
		76.7	....	....	....	....	....	....	....	....	1	....
		78.6	....	....	....	....	1	....	....	....	....	....
		79.4	....	....	....	....	....	1	....	....	....	....
		80.9	....	....	....	....	....	....	....	1	....	....
		81.1	....	....	....	....	....	1	....	....	....	....
		81.3	....	....	....	....	1	....	....	....	....	....
		83.0	....	....	....	....	....	....	....	1	....	....
		83.9	....	....	....	....	....	....	....	1	....	....
		94.2	....	....	....	....	....	1	....	....	....	....
		96.9	....	....	....	....	....	....	....	1	....	....
		97.6	....	....	....	....	....	....	1	....	....	....
		98.4	....	....	....	....	....	....	....	....	1	....

[a] Seed from a different source, but since Marquis is a variety recently introduced and appears to be constant for all its characters, it is likely that there is no genetic difference in the resistance of these strains, for seed from these same strains planted in the spring produced less than 5 per cent of bunt.

The F_4 generation shows an average of 19.9 per cent less bunt than the F_3 parent rows, but this is undoubtedly due to seasonal fluctuations, for Hybrid 128 and Marquis showed similar differences in 1919 and 1920. A closer study of the F_4's indicates that even in these extremely susceptible segregates there is a constant difference in their susceptibility comparable to the differences in the susceptibility of the parents. This is more clearly brought out by the following figures:

	Average percentage of bunt.		Difference.
	1919	1920	
From 9 low selections	80.2	62.2	18.0
From 4 high selections	96.8	72.4	24.4
Difference between high and low	16.6	10.2	6.4

Out of the 9 low F_3 rows (between 75 and 84 per cent bunt) 5 produced the smallest percentage of the 13 rows in the F_4 generation. Out of the 4 high F_3 rows (between 94 and 99 per cent bunt) selected, one produced the highest amount of bunt in the F_4 generation, and two of the others produced more than the average of all the F_4's in 1920. This indicates that Marquis carries a weak factor or property for resistance that is inherited and is not affected by fall planting. If this be true, strains from this cross could be selected that would show a consistent difference of from 15 to 30 per cent when planted in the fall under conditions favoring maximum infection. In 1919 Hybrid 128 produced 98 per cent, Marquis produced 75 per cent, and the F_3 varied from 75 to 100 per cent. In 1920 the parent varieties produced 30.9 and 74.3 per cent respectively, an F_2 progeny produced 56 per cent of bunt, and the F_4 selections varied from 46 to 85 per cent. Five plants, each selected from the lowest and highest F_4 row, produced an average of 78 and 91 per cent bunt in the F_5 generation in 1921. These figures include only the rows that were planted together in the same part of the field, and on the same date. There is much work to be done to establish the inheritance of the resistance of Marquis, especially the part played by the winter rest period, the segregation of the units that are not so affected, and the complex of its resistance for spring planting. This is being done and will be reported later.

There remains one other cross to discuss, that of Fortyfold × Red Russian. The average susceptibility of the parent varieties and their F_3 offspring in 1920 was 36.7, 49.3, and 37.5 per cent, respectively. The F_3 segregates varied from 8.1 to 73.6 per cent. Reference to figure 1 shows the greatest class frequency of the 181 F_3 rows to be between 30 and 40 per cent, with rapidly diminishing class frequencies above and below. The most striking thing about this cross is that the average of the F_3 generation is less than the average of the parents. The fact that the parents were duplicated so that there were nearly 200 plants counted precludes the possibility of a chance error. In the other four crosses charted in figure 1 the average of the parents lie below the average susceptibility of their respective F_3's, showing dominance of susceptibility. In this cross there seems to be a slight dominance of resistance, the parents producing on the average 5.6 per cent more bunt than the F_3 progeny.

The lower limit is decidedly lower than any recorded example of either parent under similar conditions. This shows that Fortyfold and Red Russian (which are known to be intermediate in respect to resistance of bunt, when compared with wheats like Turkey, Alaska, and Florence on the resistant side, and Hybrid 128 and Jones Winter Fife on the susceptible side) possess different kinds of resistance which are cumulative in effect, when brought together by crossing. These resistances have a value somewhere between 10 and 20 per cent in reducing the amount of bunt that would be produced on the extremely susceptible varieties when planted under conditions favoring maximum infection. By crossing them, segregates are obtained that are from 10 to 20 per cent more resistant than either parent. It is to be expected, and the evidence points to the probability, that segregates also occur which are from 10 to 20 per cent more susceptible than either parent. The difficulty of isolating the susceptible segregates, on account of the fungus destroying the reproductive organs, was pointed out in another place. The segregable dilute or feeble resistance of Fortyfold shown here is in accord with that found in the cross between it and Turkey. The feeble resistance of Red Russian has not as yet been definitely corroborated in other crosses.

DISCUSSION AND CONCLUSIONS

The investigation described in this paper forms a part of the general work of the Washington Experiment Station looking toward the discovery of methods for reducing the destructiveness of bunt or stinking-smut in wheat. The particular phase treated is the development of a commercially desirable wheat which will not develop bunted heads even on a badly infected soil. Emphasis has been placed on inheritance of bunt resistance, but the ultimate practical application of the principles involved has been continually kept in mind. (Pl. 3, B.)

The first tests for resistance were made in 1914 when a few of the common commercial varieties were tested for comparative resistance under conditions favoring maximum infection. During the next two years, while these and other varieties were being tested in greater detail, methods of infecting the seed, recording the data, and computing the resistance on a comparative quantitative basis were developed and standardized. In an endeavor to obtain the best possible material for the breeding work, more than 500 named varieties, from the principal wheat districts of the world, including representatives from the eight species, have been tested for resistance.

Most of the varieties tested proved to be either very susceptible or lacking in winter hardiness. Thus the representatives of the four species, einkorn, polish, spelt, and emmer, although all very resistant, were discarded because they were of no commercial value, either from the standpoint of yield or hardiness. One poulard (Alaska) was used as the male parent in several crosses, mainly because of its bunt-resisting qualities. One club (Hybrid 128) was selected for two of the crosses described below because of its stiff straw, early maturity, and prolificacy, notwithstanding its malignant susceptibility to bunt. The other six varieties were selected for the breeding work from the vulgare group because they were common commercial varieties (with the exception of Florence, which was selected for its exceptional resistance) in the Northwest, and contained the greatest range of desirable characteristics.

Alaska, Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones' Winter Fife have the winter habit of growth; that is, they will not head out if planted in the spring, and are considered sufficiently winter hardy for fall sowing. This is of prime importance because the chief losses due to bunt occur in winter wheat. Marquis and Florence are spring wheats and are not considered sufficiently hardy for fall sowing, but were such good milling wheats and so resistant to bunt that they were used for the additional purpose of studying the inheritance of winter hardiness.

These eight varieties (Pl. 2, B; 3, A) have been grown as pure lines from individual plant selections for 6 to 15 years and from the first have bred true for their morphological characters and may be assumed to be constant, within the limits of soil and seasonal fluctuations, in their comparative resistance to bunt.

Turkey is one of the most resistant of all the wheats tested, seldom producing above 5 per cent of bunted heads, even under the most favorable conditions for infection. From one-fourth to one-half of the plants may show infection, but only a few flowers, or at most only part of the heads on an infected plant produce smut balls, the remainder producing normal wheat. The other resistant varieties, Alaska, Florence, and Marquis, show similar characteristics in that only a small part of the flowers on the infected plants are destroyed by the fungus. The two very susceptible varieties, Hybird 128 and Winter Fife, on the other hand, do not normally produce anything but smut balls on the infected plants, the number of flowers or heads escaping being negligible compared with those that are destroyed. An occasional plant escapes infection altogether and produces normal grain; but once the parasite establishes itself within the host, more than 80 per cent of the plants on the average produce only the fruiting bodies of the parasite, and the others produce less than 40 per cent of grain. Fortyfold and Red Russian, which are intermediate in respect to total bunt produced, are also intermediate in respect to the amount of wheat produced on the infected heads.

The resistance of Turkey is different from that of Alaska, for segregates of a cross betwen them occurred which were more resistant than either parent, inasmuch as no trace of bunt could be obtained from the F_3 to the F_7 generation, although planted under conditions favoring maximum infection—conditions under which both parents showed traces of bunt on more than one-fourth of the plants. Such a result would be impossible unless the resistance of the two wheats were cumulative in effect, each contributing something that the other lacked. For if the resistance were the same in both parents, the offspring would fluctuate around the same mean and no segregates would occur more resistant or more susceptible than the parents. If, however, the resistance of Turkey and Alaska were due to different causes, or perhaps the genes located in different chromosomes, then the offspring would show greater variation than the parents, and segregates would occur which would be permanently more susceptible than either as well as more resistant. If the resistance of each parent were composed of differing multiple factors, many new and different segregates would occur. Thus, if Turkey resistance *abc* met Alaska resistance *xyz*, and each factor reduced the amount of bunt by 20 per cent, a cross between them should give varients (assuming the susceptibility of the parents separately to be 5 per cent) in the F_2 generation that would produce bunt as follows:

Number of resistant factors.	Bunt produced.	Classification.	Number of resistant factors.	Blunt produced.	Classification.
	Per cent.			*Per cent.*	
0	65	Nonresistant.	3	5	Very resistant.
1	45	Slightly resistant.	4	−15 0	Immune.
2	25	Somewhat resistant.	5	−35 0	Do.
			6	−55 0	Do.

Unfortunately the large number of sterile plants and flowers produced by crossing Turkey and Alaska, combined with the lack of winter hardiness, reduced the number of plants to such a degree that an accurate analysis of the particular type of segregation that occurred was impossible.

Florence is a very resistant spring wheat, but its resistance is also different from that of Turkey, for transgressive inheritance occurred in a cross between them, F_3 segregates occurring all the way from completely immune to completely susceptible individuals, not unlike the illustration given above. More than 40 per cent were immune, while the plants in 12 rows (7 per cent of the F_3 progeny) were more than 50 per cent bunt, an amount never produced by either parent under the most favorable conditions for infection. Something like 30 per cent of the rows were intermediate, producing from 10 to 50 per cent of bunted heads.

It is not too much to suppose that the 72 immune rows differed in the intensity of their resistance, but there is no way of proving it except by the slow, laborious process of crossing them with susceptible varieties and testing the comparative resistance of their descendants. The intermediate and susceptible segregates indicate multiple factors for resistance in both Turkey and Florence, the loss of which renders a given hybrid offspring in a later generation a congenial host for the parasite. Such factors added together are cumulative in effect, making the segregate possessing them immune against all attempts of the organism to set up a pathogenic relationship.

Fortyfold has a dilute or weak resistance that is evidently different from that of any of the resistant elements of Turkey, for in the F_3 generation of Fortyfold × Turkey five out of 593 F_3 families occurred that were distinctly more resistant than Turkey, and many occurred more susceptible than Fortyfold. This dilute resistance of Fortyfold showed a value of approximately 5 per cent in 1920 when added to the resistance of Turkey in the very resistant F_3 families, but in the absence of it and also the Turkey resistance, the segregates increased 15 to 20 per cent in the amount of bunt produced. High and low selections are being tested in the F_4 generation in 1921 to determine the correctness of these assumptions, for the data from the F_3 progeny are not as conclusive as one would like to have in a characteristic which shows such wide fluctuations as bunt resistance.

Hybrid 128 has no heritable resistance distinct from that of Turkey or Marquis, for the F_3 progeny of crosses with them did not give variations exceeding the extremes of the parent types. The intermediates in the F_3 generation of Turkey × Hybrid 128 indicated that the resistance of Turkey was made up of multiple factors, which, when separated, gave dilute resistances. The 334 F_3 families of Hybrid 128 × Marquis were all very smutty, none having less than 70 per cent of bunt. In the F_4 generation, however, the high selections gave 10 per cent more bunt than the low ones, which indicates a dilute resistance, probably of Marquis, that is not affected by the winter weather. Marquis is very resistant when spring sown, but this resistance is mostly destroyed or neutralized by the winter rest period when fall sown.

The resistance of Red Russian is not well established in the cross with Turkey on account of the small numbers and the unfavorable season in which it was tested, but in so far as the F_4 selections tell anything they indicate that any resistance it may possess is not different from that of Turkey, for none of the segregates were more resistant than Turkey, and the most susceptible did not surpass Red Russian in amount of bunt produced. The cross between Fortyfold and Red Russian, however, prove that the latter has a definite heritable resistance which, added to the unlike resistance of Fortyfold, produces segregates much more resistant than either parent. That is, the two weak resistances when brought together have the effect of a strong resistance comparable to that of Turkey or Alaska. The feeble or weak resistance of Fortyfold is in accord with that found in the cross between it and Turkey. The dilute or feeble resistance of Red Russian has not as yet been corroborated in other crosses in sufficient detail to warrant discussion.

From the foregoing evidence, based on seven years' work with bunt resistance in wheat, and with special reference to eight varieties, and the inheritance of resistance in the progeny of crosses between them, the general conclusions may be summarized as follows:

The most susceptible wheats, planted under conditions favoring maximum infection, produce an average of about 80 per cent of bunted heads. Hybrid 128 and Jones Winter Fife belong to this class. Although they produce 20 per cent of sound heads, this seems to be due to accident, for in crosses with other varieties the descendants do not show evidence of having inherited any cumulative resistance whatever from these varieties.

Fortyfold, Red Russian, and Marquis each have differing dilute resistances which reduce the amount of bunt by 10 to 25 per cent. When added together, as in descendants of crosses between them, the cumulative effect makes a more concentrated resistance, having a value of from 30 to 60 per cent. In addition to the dilute resistance, Marquis has a strong winter-sensitive resistance with a value of 50 to 60 per cent in spring-sown grain which is ineffective in preventing bunt when the seed is fall sown. That is, if Marquis is sown in the fall, only the dilute resistance is operative, for it produces but 10 to 20 per cent less bunt than the most susceptible varieties; if sown in the spring, the strong winter-sensitive factor becomes functional, and the resulting crop produces 60 to 75 per cent less bunt than the completely susceptible varieties. There is some evidence that all the different resistances are somewhat winter-sensitive, for the facultative wheats (those that may be sown either in the fall or in the spring, being true spring wheats but fairly winter hardy) are known to produce more bunt from fall seedings. Florence also registers a higher degree of bunted heads in the fall-sown tests.

Turkey, Florence, and Alaska each have differing concentrated resistances which reduce the amount of bunt 70 to 75 per cent, compared with the standard susceptible varieties. These concentrated resistances are also cumulative in effect when brought together by crossing, the resulting descendants segregating into immune, very resistant, various stages of dilute resistant, and completely susceptible classes.

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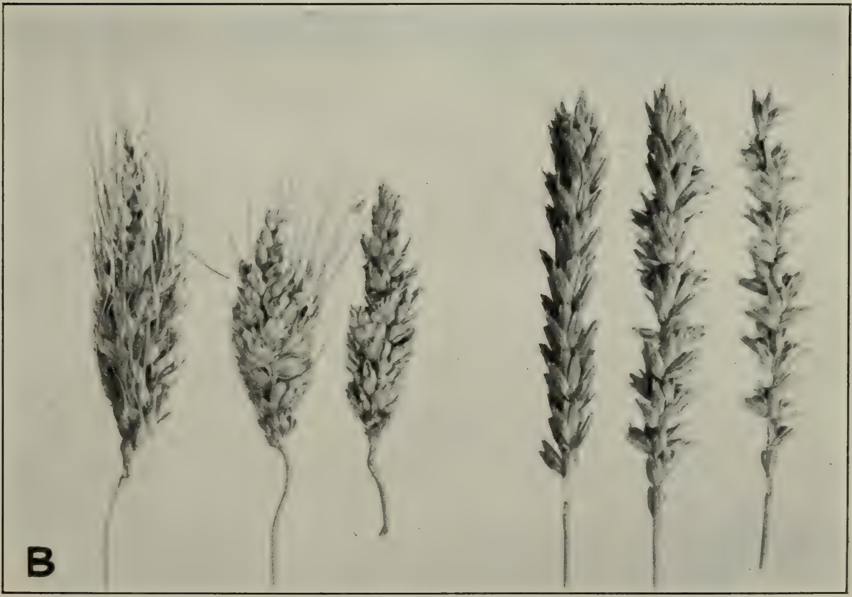
PLATE 1

A.—Result of a smut explosion. Machine of W. J. Grier, near Colfax, Wash., a few hours after the explosion, August 12, 1915. By hard fighting the sacked grain on the right was saved, but the machine and straw stack were burned. Hundreds of thrashing outfits and thousands of bushels of wheat have been destroyed in the Pacific Northwest due to fires following explosions of smut dust (bunt spores), which burn like powder.

B.—Morphological effect of bunt on wheat heads. The three on the left are Alaska, the others Fortyfold. Heads of each variety from left to right contain normal seed, both normal seed and smut balls, and only smut balls. In Alaska the parasite causes the awns to fall but dwarfs the head instead of stimulating increased length as in Turkey and Hybrid 128. (See Pl. 2, A.) The head length of Fortyfold is not affected, but the spikelets on the infected heads stand out more nearly at right angles to the rachis.

A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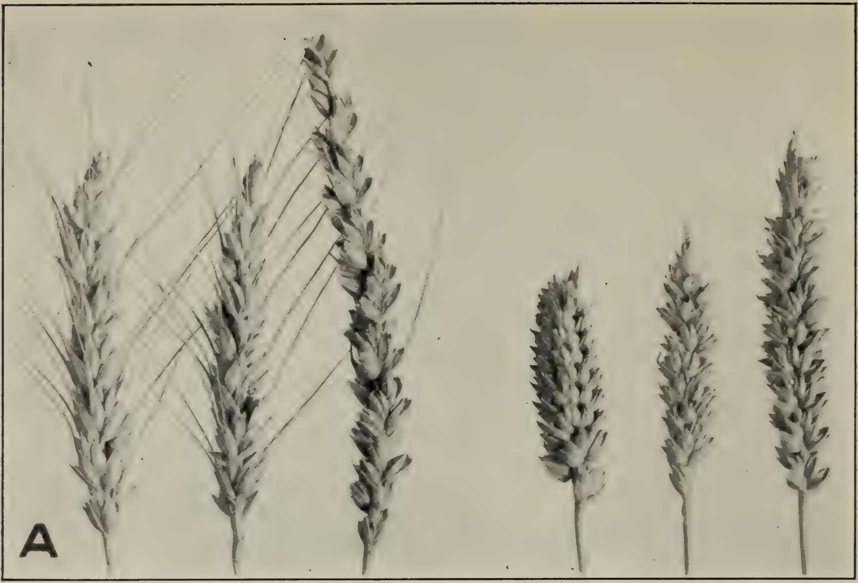
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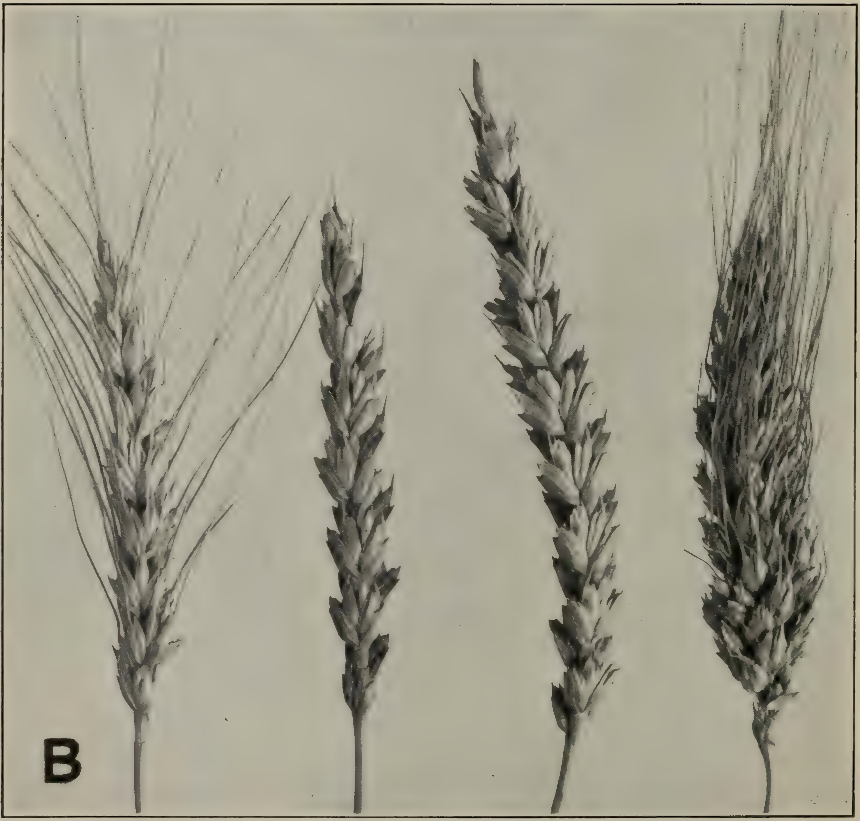
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PLATE 2

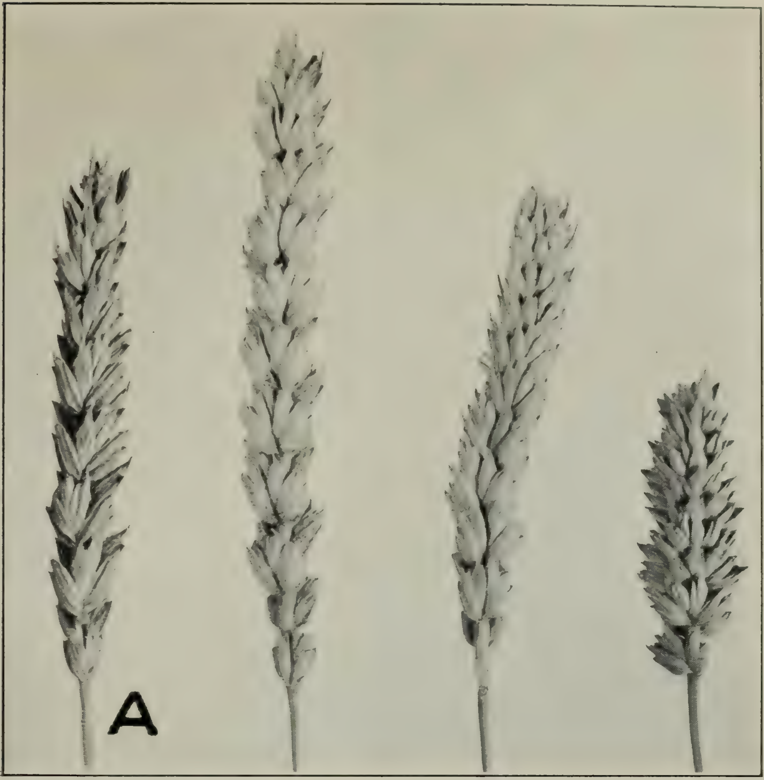
A.—Morphological effect of bunt on wheat heads. The three on the left are Turkey, the others Hybrid 128. First head on the left, normal grain; second head, half wheat and half bunt; third, all bunt. The bunted heads are much elongated as if trying to grow away from the fungus. In Turkey the awns fall off prematurely on the bunted heads. The bunted head of Hybrid 128 on the extreme right looks like a vulgare instead of a compactum.

B.—Parent varieties resistant to bunt. From left to right, Turkey, Florence, Marquis, Alaska. Turkey and Alaska are true winter wheats and can not be used for spring sowing. Florence and Marquis are typical spring wheats. Marquis and Turkey are the most popular varieties in the United States and Canada, measured by the quantity produced. Both are excellent breadmaking wheats.

PLATE 3

A.—Parent varieties susceptible to bunt. From left to right, Fortyfold, Jones Winter Fife, Red Russian, Hybrid 128. Jones Winter Fife and Hybrid 128 are about 20 per cent more susceptible than the other two. All are true winter wheats of commercial importance in the Northwest. Fortyfold and Hybrid 128 have white grain.

B.—Two hybrid (F_5) types with commercial possibilities. Left, the morphological characters of the Hybrid 128 parent are combined with the resistance of Turkey. On the right the Florence head type is combined with the winter hardiness of Turkey. It has been immune to bunt for three years. Both selections outyielded the parents in 1919 and 1920.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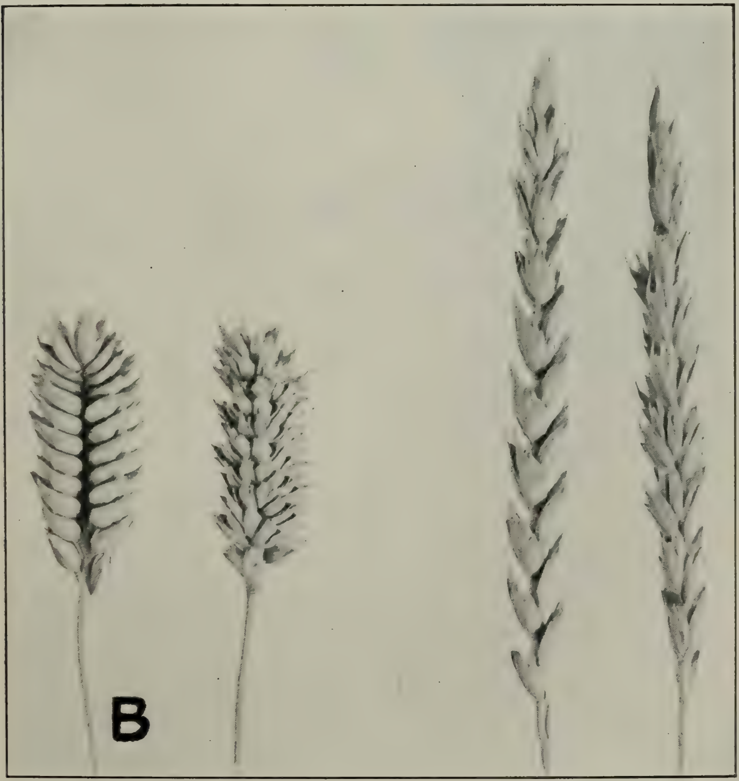
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STUDIES ON SELF-STERILITY VI. THE GENETIC BASIS OF CROSS-STERILITY IN NICOTIANA

EDGAR ANDERSON

Missouri Botanical Garden, Saint Louis, Missouri

Reprinted from GENETICS 9: 13-40, January 1924

GENETICS

A Periodical Record of Investigations Bearing on
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All subscriptions, notices of change of address, remittances, and business correspondence (except concerning volumes 1 to 6) should be sent to GENETICS, Menasha, Wisconsin, U. S. A., or BROOKLYN BOTANIC GARDEN, Brooklyn, New York. All remittances should be made payable to GENETICS.

Entered as second-class matter August 31, 1922, at the postoffice at Menasha, Wisconsin, under the act of March 3, 1879.

STUDIES ON SELF-STERILITY VI. THE GENETIC BASIS OF CROSS-STERILITY IN NICOTIANA[1]

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Received June 23, 1923

TABLE OF CONTENTS

PAGE
INTRODUCTION 13
Materials and methods 14
Review of recent literature 16
Presentation of data 20
  Nicotiana Forgetiana 20
  Nicotiana alata Lk. and Otto var. *grandiflora* Comes 24
  Nicotiana Forgetiana × *N. alata* 24
    Cross-pollinations between the families 34
DISCUSSION 34
  Working hypothesis 34
  Number of classes 36
  Hereditary nature of classes 37
SUMMARY 37
  Tabular comparison with other work on self-sterility 38
LITERATURE CITED 39

INTRODUCTION

The first papers of this series, reporting work done at the Bussey Institution of HARVARD UNIVERSITY between 1910 and 1917, left unsettled a number of questions relating to the genetic behavior of self-sterile plants. It will be remembered that studies of hybrids between *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes[2] and *Nicotiana Forgetiana* (Hort.) Sand. revealed the presence of cross-sterility of surprisingly regular behavior. Each of the populations studied could be divided, by means of its cross-sterility relationships, into a number of inter-fertile, intra-sterile classes. This may be somewhat clearer if we take a concrete example. The population known as family H was found to consist of three inter-fertile, intra-sterile classes which were arbitrarily named A, B and C. All of the members of each class were sterile with all the other members of that class but were fertile with the members of the other two classes. No plant in class A, for example, would set seed when pollinated with pollen from another plant in that same class, though it would set an abundance of seed when pollen from plants in classes B and C was applied. In other words, each class behaved as if it were a single self-sterile individual.

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[1] One of a series of papers submitted to the faculty of the Bussey Institution of HARVARD UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Science.

[2] Hereafter *N. alata grandiflora* will for convenience be referred to as *N. alata*.

Upon examining the material histologically it was found that pollen germinated normally when applied to the stigmas of the plant on which it was produced and that the pollen-tubes grew down the style at a constant rate of speed. Under normal conditions this growth was not fast enough to allow them to reach the ovule during the "life" of the flower, and therefore no seed was obtained. Precisely the same thing occurred when the pollen was applied to the stigmas of other plants in the same class. When, however, it was applied to the stigmas of plants with which it was cross-fertile, the pollen-tube grew at an increasing rate and reached the ovule in time to cause fertilization and subsequent seed production. These phenomena were interpreted as due to the interaction of various germinal factors. According to this view, plants which were cross-sterile with each other had certain essential factors in common; plants which were cross-fertile with each other differed for some of these factors. If two plants, and hence the pollen-tube and the style, differed for these factors, a stimulus was produced which caused the accelerated growth of cross-fertile matings. When, however, as in a self-pollination or a cross-sterile pollination, the pollen-tube and the style had the same factors, there was no stimulus, no accelerated growth, and fertilization did not occur. A class, according to this view, was made up of a number of plants possessing certain essential factors in common. The present investigation was undertaken to determine the number of distinct classes in the material at hand and to study the manner of their inheritance. While it was not certain that the same system of inter-fertile, intra-sterile classes operated in *N. alata* and *N. Forgetiana* as in their hybrids, there were reasons for believing this to be the case. It was planned to determine this definitely; to find out if there were any classes common to both species; and to investigate the relations between the classes of the two species and those of their hybrids.

MATERIALS AND METHODS

Owing to the exigencies of war work, much of the seed from the earlier cultures on hand was dead when the present writer took up the problem in April 1919. Because of this unfortunate but unavoidable circumstance the work could not be prosecuted as rapidly as had at first been thought possible and it was some time before the project was well under way.

Two species of Nicotiana were used, *N. Forgetiana* (Hort.) Sand. and *N. alata* Lk. and Otto var. *grandiflora* Comes. As has already been explained by EAST (1913), the *N. Forgetiana* used throughout the work with self-sterility had its origin in seeds obtained from SANDER AND SONS under the name of *N. Sanderae. Nicotiana Sanderae* (*N. alata*×*N. Forgetiana*) is very variable in color, the flowers ranging from white through pink and magenta to purple and crimson. In size they vary greatly, although they are uniformly larger than those of *N. Forgetiana*. The plants here discussed were constant through five generations for flower size, color and form and showed not the slightest trace of *N. alata* characteristics. They were, moreover, the exact duplicates of SANDER'S figure of *N. Forgetiana* published in the Botanical Magazine (No. 8006) and we therefore feel justified in supposing that they originated from seeds of pure *N. Forgetiana* which had been inadvertently mixed with the hybrid *N. Sanderae* seed.

The material was well suited to our needs. It grew splendidly under greenhouse conditions and plants could be brought into flower at practically any season of the year. Single plants could be cut back and made to flower again and again. Ninety-five percent of compatible matings were successful and there was an abundance of seed from each mating. The chief advantage for the present investigation was that the germinal factors affecting self-sterility and self-fertility were much stronger than the environmental ones. It was therefore excellent material in which to attempt to trace the course of germinal factors. Had it been otherwise, cramping the plants in pots and growing them under artificial conditions might have so obscured the effect of the germinal factors that they could not have been studied. Or if, as in many other species, the normal percentage of successes in compatible crosses had been only 50 to 75 instead of 95 it would have been much more difficult to determine whether any two plants were cross-fertile or cross-sterile.

Every precaution was taken to reduce the possibility of experimental error. All seed was planted in sterile soil. All pollinations were made upon emasculated flowers which were inclosed in paper bags. During the last few months' work when large numbers of plants were being classified, these precautions were not observed, but this was during the winter when there were no insects in the greenhouse. At all other times pollen was used only from newly-opened, bagged flowers. Whenever there was any reason to believe that pollen from nearby plants might be a source of contamination all these flowers were destroyed before the paper bag was removed. In emasculating and pollinating, the hands and instruments were washed in 95 percent alcohol after each operation to destroy any foreign pollen. The efficacy of this method was tested in the following manner; The fingers were dusted with pollen and four pollinations were made. The hands were then rinsed very lightly with alcohol. When the remaining pollen (of which there was a liberal amount) was thoroughly dry, four additional pollinations were made on the same plant. No seeds were produced from these later pollinations, but all those made before the application of alcohol resulted in plump, well-filled capsules.

The work was done under the direction of Professor E. M. EAST to whom the writer is indebted for advice and encouragement. He is also under obligations to Miss DOROTHY MOORE for assistance with the manuscript.

REVIEW OF RECENT LITERATURE

Since the earlier papers of this series were written several interesting articles on the subject have appeared. BAUR (1919) investigated the self-fertility of several species of the Antirrhinastrum section of the genus Antirrhinum and corroborated many of EAST and PARK'S (1917) observations. *Antirrhinum siculum* and a wild Spanish form of *A. majus* were fully self-fertile and set seed as well with their own pollen as when cross-pollinated. *A. tortuosum*, *A. latifolium*, and two wild forms of a Portuguese species were found to be self-sterile during the first year, although they were slightly self-fertile toward the end of the blooming season in the fall, a condition paralleled in Nicotiana by the end-season fertility reported by EAST and PARK. Unlike Nicotiana, however, these species did not regain their self-sterility with the advent of another blooming period but were very self-fertile the second year. *A. Ibanyezii*, *A. molle*, *A. glutinosum*, *A hispanicum*, and an undetermined Spanish species were found to be fully self-sterile.

Like COMPTON (1912) and EAST and PARK (1917) BAUR found that crosses between self-sterile species gave self-sterile hybrids and that in crosses between self-fertile and self-sterile species, self-fertility behaved as a dominant. BAUR obtained an F_2 which suggested a 15 : 1 ratio and this conclusion was further borne out by the fact that the self-fertile plants differed among themselves in the expression of their self-fertility, suggesting the presence of a two-factor difference. While EAST and PARK obtained a 3 : 1 ratio, there were certain differences among the self-sterile plants which suggested an additional factor; in other words a 12 : 3 : 1 ratio, a result quite in accord with BAUR'S. While BAUR was not able to investigate cross-sterility as extensively as EAST and PARK (due in part to the nature of his material), his findings are similar. In a cross between two plants of *A. hispanicum* 31 seedlings were obtained. These could be divided by their cross-sterility and cross-fertility relationships into two inter-fertile, intra-sterile groups, approximately equal in size. One group was fertile with both parents, one sterile with one parent but fertile with the other. This is remarkably like the result reported in the present paper, obtained by crossing two plants of *N. Forgetiana.*

LEHMANN (1919) studied the self-sterile species, *Veronica syriaca.* He found inter-fertile, intra-sterile classes, pseudo-fertility, and identical behavior of reciprocal crosses as regards cross-sterility. He had been conducting experiments on this species for several years. During that time he had not found a single self-fertile individual, and had noticed that certain combinations were repeatedly cross-sterile. Accordingly a cross was made between two plants, and a first generation of 198 plants was grown. All were perfectly self-sterile. Of these, 32 were chosen for intensive experiment and 983 of the possible 992 combinations were effected, 233 pollinations being made. The 32 plants could be divided into four intra-sterile, inter-fertile classes, A, B, C and D. Of the many crosses between plants of different classes, 97.5 percent were fertile; of the many between plants of the same class only 1.1 percent set seed and these were small abortive capsules. After these four groups had been determined, crosses were made with the remainder of the first-generation plants in order to classify as many of them as possible. The relationships of 131 plants were determined. They fell into four classes in the following numbers: A, 35; B, 27; C, 37; D, 31.

As *V. syriaca* is neither wild nor cultivated in the region where LEHMANN was working, it was possible to test the cross-sterility of the classes in a very severe manner by growing representatives of each class in isolated plots and allowing insects to make the pollinations. Several plants of each class were isolated, 13 A's, 10 B's, 21 C's, and 12 D's. Of the 8964 flowers recorded on these 56 plants, 8785, or practically 98 percent, set no seed. The other 2 percent for the most part formed small abortive capsules. In fact only 5 seeds were obtained from all the plants. A check plot of plants from all the classes set an abundance of seed.

DEVRIES (1906) seems to have been the first to notice inter-fertile, intra-sterile classes among self-sterile plants. In a short note on *Linaria vulgaris* quoted by CORRENS (1916), he says:

> ". . . dass es zweierlei Arten von Löwenmaul gibt, die man allerdings von aussen nicht unterscheiden kann, die aber doch verschieden sind. Bei jedem Typus sind die einzelnen Pflanzen unter sich unfruchtbar, vereinigt man jedoch zwei Pflanzen, die

verschiedenen Typen angehören, dann gibt es eine normale und reichliche Ernte."

DeVries apparently went no further with the matter and there it rested until Correns started working with the genus in 1913. He obtained 2 plants of *Linaria vulgaris* from different localities and crossed them. Unfortunately these parent plants died before the next generation came into flower, and it was possible to test the 13 plants of the next generation only among themselves. There were 4 classes, all inter-fertile and intra-sterile, except that class D was fertile with class B when used as a male parent and sterile when used as a female. The number of plants and the number of pollinations are so small that it is difficult to say whether the same sort of thing is in operation here as in Antirrhinum, Veronica and Nicotiana, or whether there are really some minor differences. Correns described the phenomenon which East (1917) has called pseudo-fertility. He also noted that several cross-sterile matings yielded small abortive capsules.

Sirks (1917) found quite a different condition in *Verbascum phoenicium*, where conditions are further complicated by the presence of true functional sterility. There was much cross-sterility but no regularity which would permit grouping into classes. Reciprocal crosses very often gave different results. There seemed to be a tendency towards dioecism and the plants could be arranged in a graded series. The extremes at one end of the series usually (about 3 times out of 4) are fertile when used as a female parent, but sterile when used as a male. At the other end are plants which seldom are fertile as females but are nearly always fertile as males.

Stout (1920) has continued his earlier work (1916) on self-sterility and self-fertility in *Cichorium Intybus* and has made preliminary investigations in several other genera. Before considering his work it will be necessary to define certain terms. If a plant sets abundant seed with its own pollen it is called self-fertile. If it sets no seed at all it is called self-sterile. In describing these two extremes all writers are in agreement. Among self-sterile plants, however, there will be found individuals which are very slightly self-fertile. This fertility may exhibit itself as a swelling of the capsule with few or no seeds produced; in only a few cases will it approach to anything like the fertility of self-fertile plants. East (1917), working on Nicotiana, showed that it was a variant of self-sterility and named the phenomenon "pseudo-fertility." A similar condition has since been reported from *Veronica syriaca* by Lehmann (1919), from *Linaria vulgaris* by Correns (1916), from Antirrhinum by Baur (1919), and Stout himself has described its occurrence in several species.

Stout has used no term to distinguish between perfect self-fertility and this pseudo-fertility and one has to judge from his data which is meant. When he says, for instance, (Stout 1917, p. 387) that 10 of 101 offspring of two self-sterile plants were self-fertile, one finds by turning to table 8 on pages 381-383 that the percentages of self-fertility of these plants are as follows: 6 percent, 13 percent, 63 percent, 3 percent, 11 percent, 50 percent, 4 percent, 71 percent and 73 percent. Not one of them is perfectly self-fertile, not more than two or three of them even approach to a self-fertility comparable with that of self-fertile plants, and yet they are all described as self-fertile! In the same way he has failed to distinguish between true cross-fertility and pseudo-cross-fertility. His failure to do this undoubtedly lies in the nature of his material. The pseudo-fertility of *Cichorium Intybus* is very variable, much more so than that of *Eschscholtzia californica*, for instance. In some cases it very nearly approaches to true fertility,—just how nearly cannot be calculated, since Stout gives no complete data for crosses that are unmistakably compatible. The high percentage of failures to set seed (as compared with the percentage in Nicotiana for instance) is another factor which has undoubtedly tended to obscure the difference between true fertility and pseudo-fertility.

Stout finds no end-season fertility (or progressive increase in pseudo-fertility) in *Cichorium Intybus*. While he comes to no definite conclusions as to the inheritance of self-sterility, he seems to recognize the presence of a germinal basis. In speaking of self-fertility and self-sterility he says (Stout 1920, p. 122),

> "Nevertheless, there is a tendency to heritability in these characters. Self-fertile plants[3] appear to constitute from 0 to 10 percent of the progeny of self-sterile plants. . . . . The offspring of self-fertile plants are more likely to be self-fertile by a proportion of about 5 to 1."

He also goes on to say that this proportion can be changed slightly by selection.

In *Verbascum phoeniceum*, Stout, like Sirks (1917), found a few individuals which were almost completely sterile as males but fertile as females. Upon examination 95 percent of the pollen was found to be shrivelled and empty, showing that there is functional sterility in addition to self-sterility.

Eschscholtzia californica was found to have little pseudo-fertility. Only 1 plant out of nearly 200 grown had a self-fertility comparable with the fertility in compatible crosses. Of these plants, 128 were completely self-sterile, 43 bore small seedless pods, 24 had small pods with few seeds. There was no end-seasonal fertility.

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[3] Bear in mind that Stout includes pseudo-fertile plants under the term self-fertile plants.

Raphanus sativus was even more strongly self-sterile. Only 1 plant out of the nearly 200 grown showed any signs of pseudo-fertility.

In *Brassica pekinensis* a few of the plants showed a mid-seasonal fertility. *Linum grandiflorum* (a heterostyled species) was self-sterile and the same forms were inter-sterile.

SHULL (1923) has recently reported on the self-sterile species *Bursa grandiflora* Bois. He finds that this species is slightly pseudo-fertile and that his cultures are made up of two inter-fertile, intra-sterile classes.

PRESENTATION OF DATA

Nicotiana Forgetiana (Hort.) Sand.

Family AB (open-pollinated *N. Forgetiana*)

Nearly all the seed of *Nicotiana Forgetiana* on hand was dead; the one lot which germinated was an open-pollinated capsule which had been gathered from a field-grown plant at the end of the season. Nineteen plants were raised from this capsule and designated as family AB. One died before coming into flower. Two were strikingly different from the rest and were identical with the hybrids which had previously been made between *N. Forgetiana* and *N. alata*. It may be well to add that hybrids had been raised between *N. Forgetiana* and all the species with which it would cross and that they were all easily recognized. Inasmuch as there was every opportunity for natural crossing between these two species in the field in which the mother plants were grown, there is no reason for believing that these two plants were anything but natural hybrids between *N. Forgetiana* and *N. alata*. They were sterile with each other and fertile with all other plants with which they were crossed. The other 16 plants were all uniform. They possessed the short, deep-red flowers characteristic of *N. Forgetiana* and their corolla length accorded with that previously reported for the species by EAST (1913).

Inasmuch as the procedure followed in studying this family is typical of that followed throughout the whole investigation, it may be well to give it in some detail. In beginning the work, cross-pollinations were made between these different plants. One week after the pollinations were made the plants were re-examined and the crosses recorded as sterile or fertile as the case might be. When any two plants were found to be cross-sterile with each other on repeated trials, they were known to belong to the same intra-sterile class. Cross-pollinations of these plants with all the other plants were then made to find out if any more plants belonged to this class. In this way the class division of the entire family was worked out. Among about forty families studied in the present investigation, not one has failed to show the division into intra-sterile, inter-fertile classes first reported by EAST and PARK (1917). While there has been an occasional sterile mating between members of different classes, this is logically to be expected and will be discussed below. Among the hundreds of plants studied, however, there has not been a single case in which a plant has been perfectly and repeatedly fertile with certain members of a class and repeatedly sterile with certain other members of the same class.

TABLE 1

Cross-pollinations within family AB. (Explanation in text).

PLANT NUMBER		CLASS l	CLASS f	CLASS g	CLASS i
	Class l				
2		0–3	4–0	1–0	0–0
7		0–5	2–2	1–0	1–0
15		0–2	8–0	2–0	1–0
	Class f				
4		3–0	2–4	1–0	1–0
10		1–2	1–10	0–0	1–0
13		3–0	0–8	1–0	1–0
16		1–1	0–5	1–0	1–0
18		3–0	0–8	1–0	1–1
19		2–0	1–7	1–0	0–0
	Class g				
9		3–0	7–0	0–0	1–0
	Class i				
14		1–0	6–1	1–1	0–0

The same cross-sterility relationships that had been observed in other self-sterile species of Nicotiana were found to obtain in *N. Forgetiana*. The 13 plants which came into flower during the time that family AB was studied, fell into five inter-fertile, intra-sterile classes. One of these classes, as has already been explained, contained 2 *N. Forgetiana* × *N. alata* hybrids. Table 1 summarizes the results of cross-pollinations with the 4 *N. Forgetiana* classes. The number of sterile and fertile combinations with each class is given for each individual. The first number in each case represents the fertile matings, the second represents the sterile ones.

Plant No. 2, for instance, was in no case fertile with other members of class l and was sterile in 3 crosses with them. It was fertile with members of class f four times, with members of class g once, and was not crossed with class i. It will be seen that only two of the thirty crosses between members of the same class were fertile. In one of these only a very small capsule was produced. It might seem from the table that there were four instead of two fertile crosses. This is because each pollination is entered twice in the table, once from the male side and once from the female. The cross-pollinations made with members of family AB *outside* the family will be discussed later. Suffice it to say that they upset all expectations by showing these four classes to be distinct from any with which they were crossed, either the classes of *N. alata* or those of the *N. alata* × *N. Forgetiana* hybrids.

Family AD(7–AB × 18–AB)

For several reasons *N. Forgetiana* was not as satisfactory to work with as the hybrids between *N. alata* and *N. Forgetiana*. The flowers were smaller and not as easy to emasculate. In a problem involving thousands of controlled pollinations this is a serious hindrance. Furthermore, while plants of this species could be brought into bloom a second and third time, they did not, like the hybrids, regain anything like their former vigor. For these reasons little work was done with *N. Forgetiana*. A second generation was grown, however, from a cross between plant number 7 of class l and plant number 18 of class f. Ninety-four plants were grown, but a number of them died or became diseased before they were classified. The results on the remaining 86, known as family AD, are summarized in table 2. The plants fell into two classes of 41 and 45 plants.

TABLE 2

Summary of cross-pollinations within family AD (7–AB × 18–AB).

	NUMBER OF TIMES STERILE		NUMBER OF TIMES FERTILE	
	With class l	With class f	With class l	With class f
Class l	150	14	6	134
Class f	14	164	134	4

Results of cross-pollinations between families AD and AB

At the time when cross-pollinations were made between the two families, the parent plants of family AB were well along in their third blooming period and were not at all vigorous. Whereas, during the first blooming period they had all been well over one meter high, there were now few that approached the half-meter mark and most of them had short, unbranched flower-stalks with only a few flowers. A preliminary test showed a high degree of pseudo-fertility. It might have been expected, therefore, that the back-crosses would give a high degree of pseudo-fertility. The results are shown in table 3.

TABLE 3

Cross-pollinations between family AB and family AD.

Number of sterile matings

	CLASS l–AB	CLASS f–AB	CLASS g–AB
Class l–AD	3	0	0
Class f–AD	0	10	0
Number of fertile matings			
Class l–AD	5	10	6
Class f–AD	6	5	5

There is certainly a high degree of pseudo-fertility but there are no sterile crosses between members of different classes. A few of these plants were kept until the next blooming period. Preliminary tests showed a comparatively low degree of pseudo-fertility. Out of 10 self-pollinations only 1 set seed, and that resulted in a small capsule. Cross-pollinations between these few plants yielded the results shown in table 4.

TABLE 4

Additional cross-pollinations between families AB and AD.

	CLASS l–AB	CLASS f–AB
Number of sterile matings		
Class l–AD	4	0
Class f–AD	0	5
Number of fertile matings		
Class l–AD	0	4
Class f–AD	3	0

By themselves, these results do not give conclusive proof of the identity of classes l and f of family AB with those of family AD. The more numerous data on similar crosses are all in accord with such an interpretation.

Nicotiana alata Lk. and Otto var. grandiflora Comes

Very little was done with this species, as it was even less satisfactory than *N. Forgetiana.* When grown in pots it has very few flowers and it is not possible to make as large a number of cross-pollinations as is desirable. This condition is aggravated by the fact that because of the extremely long flowers the pistils very often become injured in bagging and unbagging the inflorescence. Furthermore, this species is much more susceptible to leaf mosaic, rendering it difficult to keep parent plants for back-crosses.

Twenty-three plants, designated as family AC, were grown from an open-pollinated capsule of *N. alata.* Not all of them came into flower and it was possible to classify only 18. As shown in table 5, they fall into 3 inter-fertile, intra-sterile classes.

TABLE 5

Cross-pollinations within family AC (open-pollinated N. alata).

	CLASS o	CLASS m	CLASS p
Class o (5 plants)	0–16	19–0	13–0
Class m (6 plants)	19–0	0–14	14–1
Class p (6 plants)	13–1	14–1	2–12

In this table the first figure represents the number of fertile matings, the second the number of sterile matings. The plants of class o, for instance, have sixteen times been sterile with each other and have never been fertile. The 19 matings between classes m and o have all been fertile. The 14 between o and p have been fertile with one exception.

To summarize the results obtained within the two species:

1. The mechanism of cross-sterility in *N. alata* and *N. Forgetiana* is the same as that previously reported for their hybrids.
2. Four inter-fertile, intra-sterile classes were found in 11 plants grown from an open-pollinated seed capsule of *N. Forgetiana.* A cross between two of these classes gave a family the plants of which belonged half to one of these classes and half to the other.
3. Three classes were found in 19 plants grown from an open-pollinated capsule of *N. alata.*

Nicotiana Forgetiana × N. alata

EAST and PARK (1917) studied the progeny of two crosses between *N. alata* and *N. Forgetiana.* Most of the present investigation is concerned with descendants of these two crosses. Diagram 1 shows the relationships between the various "families."[4] Each family is inclosed in a bracket. The capital letter above the bracket is the "short-hand" name of the family and agrees with those used in the earlier papers of this series.

CROSS NO. 2.

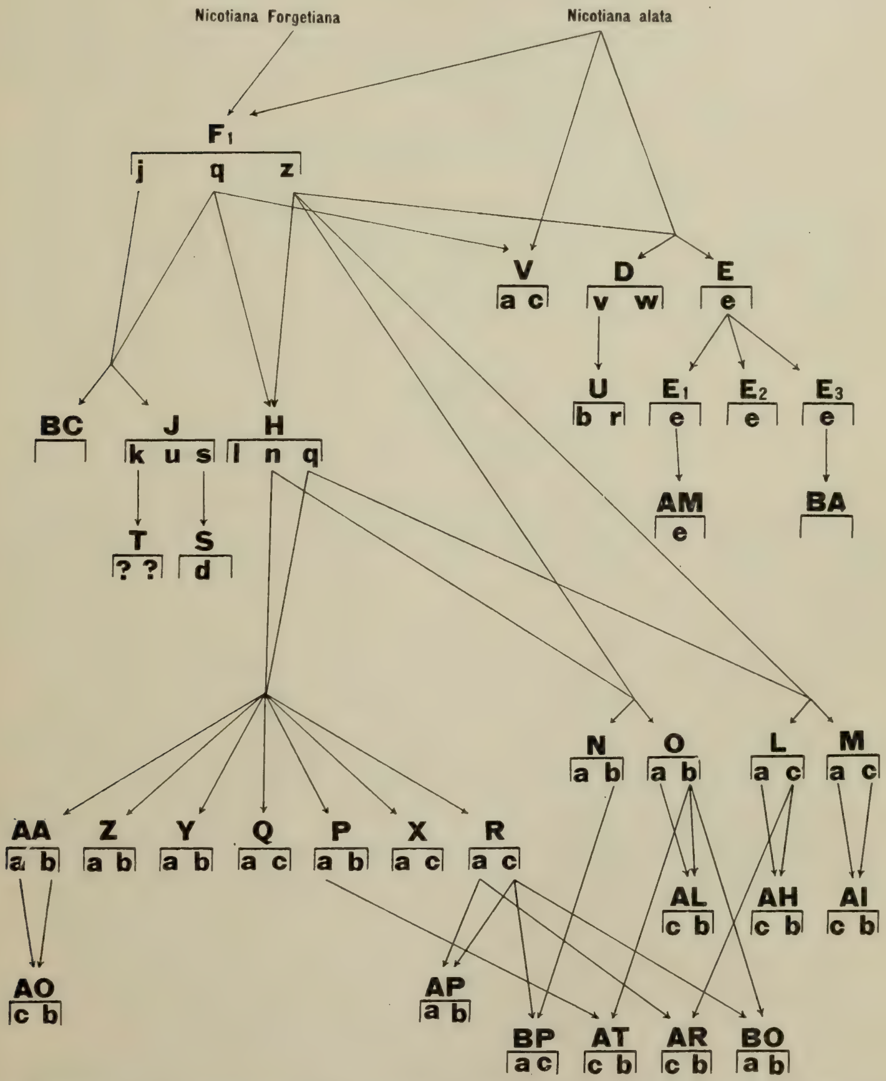

DIAGRAM 1.—The pedigree of the families of cross No. 2 discussed in this paper. The large letters designate the families, and the small letters are the symbols of the included inter-fertile, intra-sterile classes.

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[4] In the following discussion "family" means a number of plants resulting from a single cross-pollination or self-pollination.

The small letters beneath the bracket are the symbols for the various inter-fertile, intra-sterile classes. Whenever two or more classes are known to be identical in their sterility and fertility relationships they are given the same symbol. To avoid confusion through duplication the class names have been changed and do not agree with those used in the earlier papers.

The results of cross-pollinations within the families will be considered first, deferring the consideration of crosses between the families, in so far as it is possible.

Family L($H-32 \times F_1-44$)

This family resulted from a cross between a plant in family H and one of its parents. It was found to consist of two classes, a and c, containing 18 and 24 plants, respectively. The results of cross-pollinations with this family are summarized in table 6.

TABLE 6

Summary of cross-pollinations in family L.

	WITHIN FAMILY L		OUTSIDE FAMILY L			
	Class a	Class c	Class a	Class b	Class c	Class d
Sterile with class a....	76	3	14	0	2	0
Fertile with class a....	8	52	0	7	6	1
Sterile with class c.....	3	62	2	1	11	0
Fertile with class c....	52	0	5	6	0	0

Family M ($H-317 \times F_1-44$)

This family arose from a cross similar to that which produced family L. It originated from a cross between a plant in the same class of family H back-crossed to the same parent plant. It was found to contain the same two classes as family L. There were 21 individuals belonging to class a and 22 belonging to class c. Table 7 summarizes the results:

TABLE 7

Summary of cross-pollinations with family M.

	WITHIN FAMILY M		OUTSIDE FAMILY M					
	Class a	Class c	Class a	Class b	Class c	Class d	Class x	Class y
Sterile with class a...	60	4	11	0	1	11	0	0
Fertile with class a...	0	63	0	4	11	4	1	0
Sterile with class c...	4	78	3	0	15	0	0	0
Fertile with class c...	63	2	11	3	0	2	1	1

Family N (H − 373 × F_1 − 44)

Families N and O originated from back-crosses with two plants of another class of family H, and the same parent plant used in the two previous back-crosses. Family N was found to consist of two classes, a and b. There were 10 plants in class a and 7 plants in class b. Table 8 summarizes the results.

TABLE 8

Summary of cross-pollinations with family N.

	WITHIN FAMILY N		OUTSIDE FAMILY N				
	Class a	Class b	Class a	Class b	Class c	Class d	Class y
Sterile with class a...	22	2	8	0	1	0	0
Fertile with class a...	2	16	0	3	4	1	3
Sterile with class b...	2	8	0	5	0	0	0
Fertile with class b...	16	0	8	0	5	1	0

Family O (H − 335 × F_1 − 44)

Family O was found to consist of these same two classes. There were 22 plants belonging to class a and 21 plants belonging to class b. Table 9 summarizes the results.

TABLE 9

Summary of cross-pollinations with family O.

	WITHIN FAMILY O		OUTSIDE FAMILY O						
	a	b	a	b	c	d	r	x	y
Sterile with class a.........	68	3	19	0	0	0	0	0	0
Fertile with class a.........	0	68	0	9	15	1	0	3	1
Sterile with class b.........	3	42	4	10	1	0	0	0	0
Fertile with class b.........	68	2	14	0	15	6	4	2	2

Family P(H − 36 × H − 354)

Families P, Q, R, X, Y, Z and AA were the results of seven different crosses between various members of the same two classes of family H that had been back-crossed to produce families L, M, N and O. Family P was found to consist of 28 plants of class a and 17 plants of class b. The results are summarized in table 10.

TABLE 10

Summary of cross-pollinations with family P.

	WITHIN FAMILY P		OUTSIDE FAMILY P					
	a	b	a	b	c	d	r	x
Sterile with class a.........	110	7	11	1	2	0	0	0
Fertile with class a.........	0	61	0	1	11	0	0	1
Sterile with class b.........	7	14	1	6	0	0	0	0
Fertile with class b.........	61	0	16	1	10	3	1	2

Family Q(H – 324 × H – 367)

Family Q was found to consist of 13 plants of class a and 21 plants of class c. The results are summarized in table 11.

TABLE 11

Summary of cross-pollinations with family Q.

	WITHIN FAMILY Q		OUTSIDE FAMILY Q				
	a	c	a	b	c	d	x
Sterile with class a...	14	4	9	1	0	0	0
Fertile with class a...	2	29	0	5	4	1	0
Sterile with class c...	4	92	0	0	16	1	0
Fertile with class c...	29	0	16	7	0	1	3

Family R(H – 362 × H – 381)

Family R contained 17 plants of class a and 20 plants of class c. The results are summarized in table 12.

TABLE 12

Summary of cross-pollinations with family R.

	WITHIN FAMILY R		OUTSIDE FAMILY R					
	a	c	a	b	c	d	x	y
Sterile with class a.........	36	2	9	0	0	0	0	0
Fertile with class a.........	2	42	1	17	11	1	0	0
Sterile with class c.........	2	44	0	0	16	0	0	0
Fertile with class c.........	42	0	17	10	0	1	3	1

Family X(H − 381 × H − 321)

Family X consisted of 10 plants of class a and 5 plants of class c. The results are summarized in table 13.

TABLE 13

Summary of cross-pollinations with family X.

	WITHIN FAMILY X		OUTSIDE FAMILY X			
	a	c	a	b	c	y
Sterile with class a	22	0	4	0	0	0
Fertile with class a	2	3	0	7	5	1
Sterile with class c	0	0	0	0	7	0
Fertile with class c	3	0	4	4	0	0

Family Y(H − 382 × H − 354)

Family Y consisted of 7 plants of class a and 9 plants of class b. The results are summarized in table 14.

TABLE 14

Summary of cross-pollinations with family Y.

	WITHIN FAMILY Y		OUTSIDE FAMILY Y				
	a	b	a	b	c	x	y
Sterile with class a	8	1	5	0	0	0	0
Fertile with class a	0	8	0	4	4	1	1
Sterile with class b	1	20	0	5	0	0	0
Fertile with class b	8	0	9	2	3	0	0

Family Z(H − 354 × H − 373)

Family Z consisted of 5 plants of class a and 9 plants of class b. The results are summarized in table 15.

TABLE 15

Summary of cross-pollinations with family Z.

	WITHIN FAMILY Z		OUTSIDE FAMILY Z			
	a	b	a	b	c	x
Sterile with class a	12	0	6	0	0	0
Fertile with class a	0	9	0	2	2	0
Sterile with class b	0	16	0	3	0	0
Fertile with class b	9	0	8	0	2	3

Family AA(H−381 × H−322)

Family AA consisted of 6 plants of class a and 10 plants of class c. The results are summarized in table 16.

Table 16

Summary of cross-pollinations with family AA.

	WITHIN FAMILY AA		OUTSIDE FAMILY AA				
	a	c	a	b	c	d	r
Sterile with class a...	18	0	5	0	0	0	0
Fertile with class a...	0	9	1	6	4	0	1
Sterile with class c...	0	8	0	0	11	0	0
Fertile with class c...	9	0	2	2	0	1	0

Family W(F_6 of cross No. 1)

This family was a sixth-generation descendant of cross No. 1. It was found to consist of two classes, x and y, containing 13 and 18 plants, respectively. Table 17 summarizes the results.

Table 17

Summary of cross-pollinations with family W.

	WITHIN FAMILY W		OUTSIDE FAMILY W			
	x	y	a	b	c	d
Sterile with class x.....	22	2	0	0	0	0
Fertile with class x....	0	7	6	8	10	2
Sterile with class y.....	2	4	0	0	0	0
Fertile with class y....	7	0	8	2	3	2

Family S(J−473 selfed)

As has already been discussed by East and Park (1917), it is occasionally possible to self-fertilize these self-sterile plants and obtain a small capsule of seed. Two such selfings of plants in family J were grown. One of them, family T, was discarded because the plants were diseased. The other, family S, consisted of only one class, a further corroboration of East's earlier work. The results with this family are summarized in table 18.

TABLE 18

Summary of cross-pollinations with family S.

	WITHIN FAMILY S	OUTSIDE FAMILY S				
	d	a	b	c	x	y
Sterile with class d.....	116	1	0	2	0	0
Fertile with class d....	2	11	8	14	2	2

Family U(D − 175 selfed)

Family U resulted from a selfing of a plant in family D. Six plants were grown. Three of them belonged to class b and three to class r.

Family V(F_1 − 10 × *N. alata*)

Family V resulted from back-crossing one of the first-generation plants of cross No. 2 to a plant of *N. alata*. It was in a sense similar to families D and E, which had resulted from similar back-crosses. It was found to consist of 13 plants of class a and 18 plants of class c. The results are tabulated in table 19.

TABLE 19

Summary of cross-pollinations with family V.

	WITHIN FAMILY V		OUTSIDE FAMILY V						
	a	c	a	b	c	d	r	x	y
Sterile with class a.........	14	3	9	1	0	0	0	0	0
Fertile with class a.........	0	33	0	3	5	0	1	0	1
Sterile with class c.........	3	70	5	0	13	1	0	0	0
Fertile with class c.........	33	0	13	4	0	9	1	3	0

The remainder of the families were grown in very small numbers and no more crosses were made than were necessary to establish the number of classes present and to determine their identity or non-identity with the classes already studied.

Family AH(L − 18 × L − 20) was found to consist of 8 individuals of class b and 9 individuals of class c.

Family AI(M − 30 × M − 25) consisted of 9 individuals of class b and 11 individuals of class c.

Family AL(O − 32 × O − 46) consisted of 8 individuals of class b and 12 of class c.

Family BO(R − 2 × O − 2) consisted of 4 individuals of class a and 6 of class b.

Family BP(N − 7 × R − 12) consisted of 6 individuals of class a and 4 of class c.

Family AO(AA − 2 × AA − 13) consisted of 6 individuals of class b and 4 of class c.

Family AP(R − 20 × R − 13) consisted of 7 individuals of class a and 9 individuals of class b.

Family AT(P − 27 × O − 2) consisted of 11 individuals of class b and 6 individuals of class c.

Family AS(W − 1 × W − 5) had resulted from a cross between the two classes of family W, a descendant of the first cross. No parent plants were available for testing the relationships of the class of family W with those of family AS. The latter was found to consist of two classes, class dd with 8 individuals, and class df with 6.

Families AY(M − 21 × W − 11) and AZ(X − 15 × W − 17) resulted from two crosses between family W and cross No. 2. Family AY consisted of 2 plants of class sa, 3 of class sb, 6 of class sc, and 1 of class sd. Family AZ consisted of 4 plants of class ra and 5 plants of class rb.

The results for all the families are summarized in table 20.

TABLE 20

Summary of all families.

NAME	ORIGIN	CLASSES PRESENT AND NUMBER OF PLANTS IN EACH			
Family L	H–328 × F_1–44	18–a	24–c		
Family M	H–317 × F_1–44	21–l	20–c		
Family N	H–373 × F_1–44	10–a	7–b		
Family O	H–335 × F_1–44	22–a	21–b		
Family P	H–376 × H–354	28–a	17–b		
Family Q	H–324 × H–367	13–a	21–c		
Family R	H–362 × H–381	17–a	20–c		
Family X	H–381 × H–321	10–a	5–c		
Family Y	H–382 × H–354	7–a	9–b		
Family Z	H–354 × H–373	5–a	9–b		
Family AA	H–381 × H–322	6–a	10–c		
Family S	J–473 selfed	41–d			
Family T	J–489 selfed	?	?		
Family U	D–175 selfed	3–b	3–r		
Family V	F_1–10 × *N. alata*	13–a	18–c		
Family W	Cross No. 1, F_5	10–x	8–y		
Family AB	*N. Forgetiana*	7–f	4–l	2–g	1–i
Family AC	*N. alata*	6–o	6–p	5–m	
Family AD	AB–7 × AB–18	40–l	45–f		

TABLE 20 (continued)

Summary of all families.

NAME	ORIGIN	CLASSES PRESENT AND NUMBER OF PLANTS IN EACH			
Family AH	L–18 × L–20	8–b	9–c		
Family AI	M–31 × M–25	9–b	11–c		
Family AL	O–32 × O–46	8–b	12–c		
Family AO	AA–2 × AA–13	6–b	4–c		
Family AP	R–20 × R–13	7–a	9–b		
Family AR	R–38 × L–24	13–b	7–c		
Family AT	P–27 × O–2	11–b	6–c		
Family AS	W–1 × W–5	8–dd	6–df		
Family AY	M–21 × W–11	2–sa	3–sb	6–sc	1–sd
Family AZ	X–15 × W–7	4–ra	5–rb		
Family BO	R–2 × O–2	4–a	6–b		
Family BP	N–7 × R–12	6–a	4–c		

TABLE 21

Summary of cross-pollinations between classes. x=fertile, o=sterile.

a	b	c	d	e	r	l	f	g	i	m	o	p	sa	sb	sc	sd	ra	rb	ga	gb	gc	x	y	dd	df	h	
o	x	x	x	x	x	x	x		x	x		x	x		x	x	x		x	x		x	x	x	x	x	a
	o	x	x	x	x	x	x	x		x	x	x	x			x	x		x	x	x	x	x	x	x	x	b
		o	x	x	x	x	x	x	x	x		x	x		x		x		x	x		x	x	x	x	x	c
			o		x	x	x	x	x													x	x				d
				o			x				x												x	x	x		e
					o																						r
						o	x	x	x	x	x	x											x	x		x	l
							o	x	x	x	x	x											x	x	x	x	f
								o	x		x	x									x		x	x	x	x	g
									o																		i
										o	x	x												x			m
											o	x					x			x				x		x	o
												o			x									x		x	p
													o	x	x	x	x	x	x	x	x			x			sa
														o	x	x	x	x	x	x							sb
															o	x	x	x	x					x			sc
																o	x	x		x				x			sd
																	o	x	x	x	x			x	x		ra
																		o									rb
																			o	x	x			x	x		ga
																				o	x				x		gb
																					o						gc
																								x		x	x
																							o				y
																								o	x		dd
																									o		df
																										o	h

Cross-pollinations between the families

The same procedure was followed in establishing the relations between the classes of the different families as had been followed in working with the individual families. If crosses between two classes in different families were repeatedly sterile the two classes were known to be identical and were given the same symbol. These data are summarized in table 21. The data for each family have already been presented in tables 6 to 19.

If there had been any need for more proof of the reality of this system of inter-fertile, intra-sterile classes, it was given when all the classes a, all the classes b, and all the classes c, were crossed together. From the results obtained, the a's might all have been from one family, or the b's, or the c's. Each individual was sterile with all the other members of that class—no matter from what family—and fertile with members of all other classes. There were a few exceptions, to be sure, but they formed less than four percent of the cases, and none of them could be duplicated by repeating the same pollination. *There was not a single instance in which a plant was repeatedly sterile with certain members of a class and repeatedly fertile with certain other members of the same class.* Table 22 summarizes the results of these inter-family crosses.

DISCUSSION

Working hypothesis

The following were among the facts established by the earlier work on the subject:

1. Self-sterility is heritable.
2. Under normal conditions, the pollen-tubes produced by pollen from any self-sterile plant will not grow in the styles of that plant with a rapidity sufficient to reach the ovules during the "life" of the flower.
3. Similarly, under normal conditions, the pollen-tubes of this plant will not grow in the styles of any other plant with which it is cross-sterile with a rapidity sufficient to reach the ovules during the "life" of the flower.
4. Pollen-tubes from pollen of cross-fertile plants will grow with a rapidity sufficient to allow fertilization.

This work had suggested certain conclusions and from them the following working hypothesis was assumed in beginning the present investigation:

The phenomena of 2, 3 and 4 are due to the action of certain essential factors. When two plants—and hence the pollen-tube and the style—

TABLE 22

Results of cross-pollinations between families containing classes a, b and c. o indicates cross sterility; x indicates cross-fertility. a-L means members of class a of family L, a-M means members of class a of family M, etc.

a-L	a-M	a-N	a-O	a-P	a-Q	a-R	a-V	a-X	a-Y	a-Z	a-AA	c-L	c-M	c-Q	c-R	c-V	c-X	c-AA	b-N	b-O	b-P	b-U	b-Y	b-Z	
o	o	o	o	o	o	o	o			o	o	x	x	x	x	x			x	x	x			x	a-L
	o	o	o	o	o	o	o				o	x	x	x	x	x			x	x	x	x			a-M
		o	o	o	o	o	o				o	x	x	x	x	x			x	x	x				a-N
			o	o	o	o	o	o	o			x	x	x	x	x				x	x	x	x		a-O
				o	o		o	o				x	x	x	x	x				x	x		x	x	a-P
					o	o	o				o	x	x	x	x	x				x	x		x	x	a-Q
						o	o			o	o	x	x	x	x	x		x	x	x	x		x	x	a-P
							o		o	o	o		x	x	x	x		x	x				x		a-V
								o	o				x	x	x		x			x	x		x		a-X
									o	o				x	x					x	x			x	a-Y
										o				x		x			x		x			x	a-Z
											o	x	x	x	x	x		x	x		x		x	x	a-A
												o	o	o	o	o	o	o	x	x				x	c-L
													o	o	o	o	o	o	x	x	x				c-M
														o	o	o	o	o	x		x	x	x		c-Q
															o	o	o	o	x	x	x			x	c-R
																o	o	o	x	x	x	x			c-V
																	o	o		x					c-X
																		o	x		x		x		c-A
																			o	o	o	o	o	o	b-N
																				o	o	o	o	o	b-O
																					o		o	o	b-P
																						o	o		b-U
																							o	o	b-Y
																								o	b-Z

differ for these factors, a stimulus is produced which causes the accelerated growth of cross-fertile matings. When, on the other hand, as in 2 and 3, the pollen-tube and the style have the same essential factors in common, there is no stimulus and no accelerated growth. These essential factors are heritable and given suitable material, it should be possible to demonstrate their presence and the manner of their inheritance.

Number of classes

As little had previously been done in identifying the classes of different families with one another, a number of questions immediately presented themselves:

In these crosses between *N. alata* and *N. Forgetiana* are we dealing with a few classes or with a great many? Would we obtain these same classes upon making further crosses between the two species or would we obtain different ones? Is the mechanism of cross-sterility the same in the pure species as in the hybrids? Can we find any of these same classes (and hence the same factors) in *N. alata* and *N. Forgetiana?* How are these classes passed on from generation to generation?

Some of these questions can now be answered definitely: In the first place, there are many more classes than had at first been suspected. In the more of less inbred descendents of cross No. 2 between *N. alata* and *N. Forgetiana* there are at least five and perhaps six different classes, the relationships between two of them never having been determined. In the sixth generation descendants of the first cross (family W) there were two classes which were distinct from the five classes of the second cross with which they were tried. A seventh generation was grown from these plants (family AS) and was found to contain two classes. It was not possible to test them with the parent plants of family W. They were, however, different from any of the classes with which they were crossed. There were at least four classes in the few *N. Forgetiana* plants grown, and three classes in the *N. alata* plants. These seven classes were distinct from each other and from all the other classes with which they were tested. In crosses between family W and cross No. 2, seven classes were obtained. They were all different from each other, from the parent classes, and from all other classes with which tests were made.

All of these data are summarized in table 22 where the inter-fertility relationships of these 27 classes are indicated. We have good reason to believe that the two classes of family AS, dd and df, may be the same as the two parent classes, x and y, of family W. It is also quite possible that class r and class e are identical. All other cases in which nearly related families might have been expected to contain identical classes have been adequately tested. This leaves 24 classes. In so far as they have been tested, the 24 are all distinct from one another and there is little reason for believing that each one is not distinct from every other one.

In the present investigation we have grown a few more or less inbred descendants of two crosses between *N. alata* and *N. Forgetiana;* two crosses between these two strains; a few plants from an open-pollinated capsule of *N. Forgetiana;* and a few plants from an open-pollinated capsule of *N. alata*. In these few samples 24 classes have been found. It seems probable that in the two species and in the various combinations between them, there must be a great many more possible classes. If this be true it is not at all surprising that the two crosses made by EAST and PARK (1917) differed considerably as regards their class behavior.

Hereditary nature of classes

There was every indication that these classes have a genetic basis. This phase of the work is being continued and for the present the evidence may be summarized as follows:

1. Nearly related families possessed certain classes in common, while the classes of more distantly related families were all distinct from one another.
2. The same classes appeared in successive generations.
3. While the details are yet to be worked out, the classes are apparently inherited in regular Mendelian fashion.

These points are demonstrated in diagram 1. There it will be seen that all of the more or less inbred descendants of family H consisted of only three classes and that there was a certain regularity in the manner of their inheritance. Families U, V and E, less nearly related, contain some of these same classes and certain others which are distinct. The classes in cross No. 2 are distinct from those in cross No. 1, as are also the classes in *N. alata* and *N. Forgetiana*.

SUMMARY

1. The same system of inter-fertile, intra-sterile classes was found to exist in *N. Forgetiana* and in *N. alata* as had previously been found to occur in their hybrids and in other self-sterile species of Nicotiana.
2. Among the few strains of *N. Forgetiana*, *N. alata*, and *N. alata* × *N. Forgetiana* that were studied in the present investigation, there were at least 24 such classes. There must be many more in the two species as a whole. From one open-pollinated capsule of *N. Forgetiana* 4 classes were

TABLE 23

Tabular comparison with other work on self-sterility.

MATERIAL	REPORTED BY	CROSS-STERILITY	INTER-STERILE CLASSES	PSEUDO-FERTILITY	CLASSES INHERITED	STERILITY RECESSIVE IN CROSSES WITH SELF-FERTILE SPECIES
Nicotiana alata	ANDERSON (this paper)	——	——	——	——	——
N. Forgetiana	ANDERSON (this paper)	——	——	——	——	——
N. alata × *N. Forgetiana*	EAST and PARK (1917) ANDERSON (this paper)	——	——	——	——	
N. commutata	EAST and PARK (1917)	——		——		
N. commutata × *N. Forgetiana*	EAST and PARK (1917)	——	——	——		
N. angustifolia	EAST and PARK (1917)	——	——			
N. glutinosa	EAST and PARK (1917)	——		——		
Linaria vulgaris	DEVRIES (1906) CORRENS (1912–1916)	——	——	——		
Antirrhinum hispanicum	BAUR (1919)	——	——	——		——
Veronica syriaca	LEHMANN (1919)	——	——	——		
Cardamine pratensis	CORRENS (1912)	——	?	——	?	
Reseda odorata	COMPTON (1912)					——
Bursa grandiflora	SHULL (1923)	——	——	——		

obtained; from a similar capsule of *N. alata* 3 classes were obtained. These 7 classes were all distinct from each other and from classes in the hybrids.

3. These phenomena are interpreted as due to the action of certain factors. Plants which are cross-sterile with each other (which belong to the same class) have certain essential factors in common. Plants which are cross-fertile with each other (which belong to different classes) differ for these factors.

4. These factors are germinal and are passed down from generation to generation. Two lines of evidence point to this interpretation: a. The common possession of certain classes by nearly related families and the absence of any classes in common by families less closely related. b. The regularity with which these classes appear in subsequent generations.

This, then, is the status of the self-sterility problem in Nicotiana. Table 23 shows how certain of these results have been confirmed by the work of other investigators. This close corroboration justifies the suggestion that the conditions reported here may be somewhat general among self-sterile plants.

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GENETICS, JANUARY, 1924

TABLE OF CONTENTS

PAGE

Portrait of Carl Friedrich von Gärtner................Frontispiece

Castle, W. E., and Wachter, W. L., Variations of linkage in rats and mice.. 1

Anderson, Edgar, Studies on self-sterility VI. The genetic basis of cross-sterility in Nicotiana.................................. 13

Warren, Don C., Inheritance of egg size in *Drosophila melanogaster*.. 41

Mavor, James W., and Svenson, Henry K., An effect of X rays on the linkage of Mendelian characters in the second chromosome of *Drosophila melanogaster*................................ 70

FRONTISPIECE

This portrait of Carl Friedrich von Gärtner (born May 1, 1772; died September 1, 1850) is from a life-size oil-painting which with other relics of her father was bequeathed by Gärtner's daughter, Fräulein Emma Gärtner (d. June 10, 1875), to the Botanischen Institut of the University of Tübingen, Germany. We are greatly indebted to the present Director of the Institut, Professor Doctor E. Lehmann, for the photograph of this portrait, also for the autograph signature, and for information regarding the portrait and its painter.

The original of this portrait remains in the family of the great plant hybridizer. It was made in 1831 by the celebrated portrait artist Morff (b. 1771) who was Court Painter to King Friedrich of Württemberg. Morff's portraits were known for their great fidelity to nature as well as for his ability to depict the spiritual qualities of his subjects. These characteristics are well exemplified also in his portraits of Ludwig Uhland, Balthasar Haug, Schiller's teacher, of the musician, John Ammon, and of other celebrated persons.

GENETICAL ASPECTS OF SELF- AND CROSS-STERILITY

E. M. East

[Reprinted from the American Journal of Botany, X: 468–473, November, 1923.]

Reprinted from the AMERICAN JOURNAL OF BOTANY, X: 468–473, November, 1923.

GENETICAL ASPECTS OF SELF- AND CROSS-STERILITY[1]

E. M. EAST

With the exception of the war period, the genetical and physiological aspects of self-sterility in plants have been under investigation at the Bussey Institution of Harvard University for the last twelve years; and, as often is the case with material worked intensively, the experiments have opened up so many new problems that they will probably be continued for several more years to come. The opportunity afforded by this symposium for bringing together a summary account of that phase of the work having to do with heredity is very welcome, therefore, both because results as yet unpublished have thrown new light on the subject, and because the previous accounts have been too long and detailed for ready reference. The experiments to be reported on here include only those upon the genus Nicotiana which for the last three years have been carried on by Dr. E. S. Anderson, who gives me permission to refer to his unpublished records. Experiments on some other species which I started four years ago are not yet ready for publication; but it may be stated that the data from these later experiments are in no wise contradictory to what I shall have to say.

The self-sterile species used were *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, *Nicotiana angustifolia* R. and P. var. *crispa* Cav., *Nicotiana commutata* Fisch. and Meyer, *Nicotiana glutinosa* L., and a species believed to be *Nicotiana Forgetiana* (Hort.) Sand. A self-fertile species, *Nicotiana Langsdorffii* L., was also utilized.

Both *N. Forgetiana* and *N. alata* cross easily with *N. Langsdorffii*. The F_1 plants show no evidence of true sterility, *i.e.*, they form normal capsules full of seeds as readily as do either of the parent species. All F_1 plants are self-fertile; and when selfed produce F_2 populations consisting of about 3 self-fertile plants to 1 self-sterile plant. One may assume, therefore, that self-sterility in Nicotiana is due to the presence of the allelomorph of a dominant self-fertility factor, F. When a population is homozygous or heterozygous for factor F, it is self-fertile; when a population is homozygous for f, that is to say when it is ff, it is self-sterile. These results corroborate those of Compton for *Reseda odorata*, and have been strengthened by another similar investigation made by Baur on Antirrhinum.

The other three self-sterile Nicotianas used in the work, *N. angustifolia*, *N. commutata*, and *N. glutinosa*, will not cross readily with self-fertile species, hence the genetic relationship could not be determined. But from certain evidence gleaned from studying the behavior of the self-sterile plants among themselves, one is led to the belief that either the primary self-sterility factor exists in various allelomorphic forms each having a different efficacy in causing self-sterility or that secondary factors exist which modify this efficacy.

---

[1] Read in the symposium on "Sterility in Plants," at the joint meeting of Section G of the American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society, at Cambridge, December 27, 1922.

This variation in the efficiency of the self-sterility factor is a peculiar thing. It does not mean that all plants homozygous for *ff* are not self-sterile, for this is not the case. It means only that there is a considerable variation in the ease or difficulty with which environmental changes can produce the slight indication of self-fertility which I have termed pseudo-fertility. Under normal conditions all plants of these species are wholly self-sterile. When grown with ordinary care either in the greenhouse or out of doors they produce not a single seed after selfing, for the first month or so of the flowering season. Later, a few seeds will sometimes be produced after selfing, and this pseudo-fertility appears as a response to changed environmental conditions more readily in some families than others.

There has been some criticism of the use of the term *pseudo-fertility*. The critics would have it that a plant which produces any seed whatsoever after a self-pollination is self-fertile, no matter if it has shown complete self-sterility in 200 or 300 attempts at selfing under what I term normal conditions. The position of these critics is indefensible. It shows an astonishing ignorance of both genetical methods and genetical results. Furthermore, those who take this position are so handicapped by it that they can make no constructive analysis either of their own or other results on self-sterility, although the data yield to a very simple interpretation when it is understood that this pseudo-fertility is a mere environmental fluctuation having nothing to do with heredity. I might say in passing that every somatic character is affected by external conditions. In ordinary cases, such as flower color, one finds that he must make his records before the flower wilts and fades. In less common examples, like the Drosophila mutant having 12 legs, the individuals must be raised under extraordinary conditions in order to bring out the character—in this case extreme cold is necessary. Another example would be hair characters in the human race. Would these critics say that hair color could not be studied because a man becomes bald? Could it be maintained that I did not inherit brown hair, just because I do not have much of it left now, or because of the prospect that I will not have any left a little later? I propose to sit tight on this proposition against all-comers as a matter of honor.

Speaking seriously, pseudo-fertility in the self-sterile plants with which I have been working is a rare occurrence; but it may be caused by any proper combination of circumstances which tends to make the flowers hang to their stems longer and which tends to make the pollen tubes grow more rapidly. Concretely, it is found when the plants are old and the temperature is between 80° and 90° F.; but these conditions produce more marked effects on some families than on others. Since there is no difficulty in distinguishing true self-fertility from pseudo-fertility, the phenomenon has its advantages. By pollinating young buds of old plants under proper temperature conditions, one can obtain selfed capsules containing between one tenth and one half of the normal complement of seeds, and thus one can deal with populations produced by self-fertilization in self-sterile strains.

The most interesting problem of self-sterility is the behavior of self-sterile plants when crossed among themselves. Darwin supposed each plant to be so specialized in its reproductive organs that, though it could not be fertilized by its own pollen, it could be fertilized by the pollen of any other plant of the same species. Such a particularized specialization he found difficult to explain. Fortunately it is not necessary to explain it, for it is not what actually happens. The self-sterile strains are made up of groups of plants wherein each individual is cross-sterile with all other plants of the same class and cross-fertile with all plants belonging to any other class. In other words, if plant *A* is sterile with plant *B* and with plant *C*, it may be predicted that plant *B* will be sterile with plant *C*; and if plant *A* is fertile with plant *X* which in turn is sterile with plant *Y* and plant *Z*, then it may be predicted that plant *A* will be fertile with plant *Y* and plant *Z*. I shall not discuss the physiological implications of this peculiar phenomenon, but shall describe briefly the facts emerging from the pedigree-culture experiments.

Perhaps the most important genetic question is whether these classes of plants, wherein the individuals are all sterile with each other, are classes which are analogous to the classes of purples, magentas, reds, pinks, and so on, found in the F_2 generation after a white sweet pea is crossed with a purple sweet pea. That is to say, it is desirable to know whether one is dealing with a case of straight inheritance comparable with other cases of inheritance, but where the members of the classes, instead of being distinguishable by visual methods, can be ticketed and grouped only by the criterion of cross-sterility.

To determine this, a population of plants from a cross between *N. Forgetiana* and *N. alata* was tested for cross-sterility. Only 2.4 percent of cross-sterility was found, a percentage so small that it is not difficult to see how easy it was for Darwin to be misled into thinking that every plant within a self-sterile species was cross-fertile with every other plant. Though computation shows that from 20 to 25 intra-sterile, inter-fertile classes would account for such a small percentage of cross-sterility in random crosses, it is obvious that with such a slow-going means of testing the affinities of each individual a clear analysis of a population containing so many groups is impracticable. If the behavior of these self-sterile plants is governed by mendelizing factors, however, then it follows that a series of self-pollinations or of sister-brother matings for several successive generations will automatically reduce the number of segregating factors and hence the number of intra-sterile, inter-fertile classes. By taking advantage of pseudo-fertility it ought to be possible theoretically to obtain a population of plants belonging to a single class, a population in which every individual is cross-sterile with every other individual, though in this population each individual may fertilize the gametes or be fertilized by the gametes of other populations. These results, predictable on theoretical grounds, were actually obtained. A dozen or so populations with only two intra-sterile classes have been raised and studied, and two populations consisting of a single intra-sterile class have been found. Dr. Anderson has nearly completed an analysis of the precise method by which the controlling factors are inherited. It does not seem advisable for me to discuss these results in detail here as they will be published shortly, but the main facts bearing on the general genetic problem of self-sterility can be stated in a very few words.

First, the behavior of reciprocal crosses is the same. If the pollen of *A* is sterile on *B*, then the pollen of *B* is sterile on *A*. This is an important fact. In many of the populations studied, segregation and recombination of factors for pistil length was such that extremes of 25 mm. and 70 mm. were found. But under the usual conditions for carrying on the work, conditions under which pseudo-fertility was not a disturbing feature, incompatibility of the short-pistiled plant for pollen from the long-pistiled plant was just as marked as when the reverse cross was made. And, similarly, interclass crosses were just as easy to obtain when the long-pistiled plant was the mother as when the short-pistiled plant was used. Dr. Anderson believes that this result is aided by a high positive correlation between the length of "life" of the flower and the length of its pistil. Be that as it may, the growth rate of the pollen tubes in incompatible matings is so slow that even if the flower from a short-pistiled plant remained on the stem for as long a period as with its long-pistiled sister, the tube does not have the opportunity to traverse more than two-thirds of the pistil distance. It is possible, however, so to control conditions that one may sometimes obtain seed from a particular mating when it is impossible to obtain it after a mating made the reverse way. For example, if plant *A*, a plant in vigorous condition and at the height of its flowering season, is used as the pollen parent on an incompatible plant *B*, a plant in weakened condition at the extreme end of its flowering season, some seed will be obtained as a manifestation of pseudo-fertility; but the reciprocal cross is impossible.

A further conclusion may be derived in part from the fact that reciprocal matings are identical when not interfered with by external conditions whose effects are fairly well known and for which reason are controllable and their results predictable. It is to the effect that, though the gametes formed by a particular plant may be packed with many different combinations of genes, as proven by the variable population of zygotes produced, as far as their activities before fertilization are concerned they behave as if they were all alike. Pollen grains from a single plant may be of variant constitutions with reference to their transmission of qualities to the next generation, therefore, but they show no *selective* fertilization. They are controlled in their gametophytic activities by the genetic constitution of the mother plant on which they are formed. For this reason a genetic experiment conducted in such a manner as to have the critical matings made with pollen from a single plant will give the results to be expected from *chance* matings of germ cells. But one can easily imagine types of genetic matings where the results would be vitiated by not keeping this fact in mind. Suppose, for example, that one desired to make a test mating on plant *A* with pollen from plant *B*. Plant *B* produced little pollen, however, and additional pollen from plant *C* was used because plant *C* was assumed to have the same constitution as plant *B* with regard to the particular factors under investigation. Unfortunately the pollen tubes from the pollen of plant *C* grow faster than the pollen tubes from the pollen of plant *B*, and the resulting zygotes are all sired by plant *C*. Naturally, no geneticist in his right mind would make a test in this way, but nevertheless it may be well to have in mind its possibilities.

Second, the factors which govern the behavior of self-sterile plants are strictly inherited, and are transmitted in accordance with a definite Mendelian mechanism. Class *A*, for example, is class *A* wherever found. A single class has been identified by the cross-sterility test through three successive generations by Dr. Anderson; and the same class has been found in collateral families as far removed as fourth cousins. Thus the behavior of these factors controlling a peculiar physiological difference is exactly the same as that to be found where visually identifiable morphological differences are found.

Third, the genes which govern the behavior of these plants in crosses are numerous. About fifteen classes have been proven to be genetically distinct from each other by proving *each* class to be fertile with *every* other class. Eight or ten other classes have proven to be distinct from all other classes with which crosses have been made; but since every possibility of linking them up with known classes has not been tested, it can not be maintained that they must belong to separate groups. Arguing the matter as a problem in probabilities, however, it can be said that the chances are greatly in favor of there being more than twenty genetically different intrasterile groups of self-sterile plants in *Nicotiana Forgetiana* and *Nicotiana alata*.

It is greatly to be regretted that it has been impossible to test out thoroughly a good random sample of individuals in these two species. When our work on the self-sterility problem was resumed after the war, only two packets of *N. alata* seed and one packet of *N. Forgetiana* seed would grow. The pedigrees of these seeds were such as to make it highly probable that each population had been so selected as to reduce the number of intra-sterile groups materially. And so it proved. There were only three or four such classes in each species; and the interesting thing is that no single class of one species was found in the other species or in the classes observed in the descendants of the original hybrids between them which had been made between 1910 and 1912. We can not say therefore whether *N. alata* considered as a species contains any factors governing the behavior of self-sterile plants which are the same as those possessed by *N. Forgetiana*, or not; but from the results obtained we are justified in believing that each species does contain a relatively large number of such factors. They contain such a large number of these factors that the practical result of making random crosses in an unselected population is to obtain such a high percentage of success as to make one believe, with Darwin, that each plant is fertile with the pollen of every other plant.

In conclusion I will say only this: though the study of self-sterility in detail has opened up various fruitful lines of research of which no one can see the end, and though an adequate physiological interpretation of the behavior of incompatible pollen tubes as compared with compatible pollen tubes has not been forthcoming, the genetical problem in its narrow sense, that is to say the problem of the mechanism of its heredity, may be said to be solved. The corroborations of our results coming in from Europe on other species are too exact in detail for one to feel that there is much weight in the criticism that these results refer only to four species of Nicotiana. One can only hope that they will be helpful not only to genetic theory, but to the practical problems of the orchardist who has to deal with self-sterile fruits. They do indeed show why whole varieties of asexually propagated fruits are self-sterile. And, further, the work on the causes and control of pseudo-fertility points the way to a practical method of orchard procedure.

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The Physiology of Pollen

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Submitted to the Faculty of the Bussey Institution of Harvard
University, June, 1923, in Fulfilment of the Thesis
Requirements for the Degree of
Doctor of Science.

Reprinted from The American Journal of Botany, Vol. XI. Nos. 4, 5, 6, 7,
April, May, June, July, 1924.

THE PHYSIOLOGY OF POLLEN

Reprinted from the AMERICAN JOURNAL OF BOTANY, XI: 218–228, April, 1924.

THE PHYSIOLOGY OF POLLEN
I. THE REQUIREMENTS FOR GROWTH[1]

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(Received for publication July 5, 1923)

Following the demonstration of sexuality in the higher plants by Camerarius in 1694 and his recognition of the fact that in some way the pollen serves as the male element, a period of over a century elapsed before any definite idea was gained as to just how the pollen functions in fecundation. Amici (1824),[2] while examining the papillar cells of the stigma of *Portulaca oleracea*, noticed by chance that one of the "hairs" among those he was examining terminated in a pollen grain. This was the discovery of the pollen tube. Six years later, the same author (Amici, 1830) observed and described the formation of pollen tubes on the stigmas of Hibiscus and the gourd, and traced them through the tissue of the style to the ovary. Amici recognized that the pollen tube as a definitely organized structure grows down through the style. He further saw that one pollen tube passes to each ovule.

Brongniart (1827) had contended that the fertilizing element is transported to the ovary in the interstices of the conducting tissue of the style, without making out, however, just how this is accomplished. Schleiden (1837) and Schacht (1851, 1858) ascribed to the pollen tube the major rôle in the formation of the embryo, regarding the ovule as merely serving to enclose the tip of the tube which itself became the embryo. The careful researches of Hofmeister (1849) finally settled the bitter contention that arose regarding the respective rôles of the pollen tube and ovule in embryogeny by demonstrating clearly that, as Amici had maintained, the embryo arises from an egg within the ovule and that the pollen tube serves to stimulate this egg to development. When Strasburger (1884) described fertilization in the higher plants as consisting essentially of the union of the egg nucleus with a nucleus brought in by the pollen tube, the real significance of the pollen-tube growth in the style was first clearly appreciated. The rôle of the second male nucleus brought in by the pollen tube was cleared up when Nawaschin (1899) and Guignard (1899) independently discovered its fusion with two polar nuclei to form the primary endosperm nucleus.

In answer to his own question as to how tubes arising from the small pollen grains are able to traverse long styles, Amici (1830) advanced the view that the tubes derive nourishment from the stylar tissue in their growth toward the ovary.

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[1] Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

[2] References are to literature cited at the end of Part IV, to appear in a future issue of this JOURNAL.

That pollen of one species might form tubes in the styles of very different forms was established by Strasburger (1886). In the styles of *Convallaria latifolia*, for example, it was found that pollen from *Fritillaria persica* formed tubes that grew the whole length of the style to the ovary. These failed, however, to enter the micropyle. Pollen of Agapanthus, a monocotyledon, germinated on the stigma, and grew readily in the styles, of *Nicotiana tabacum*, a dicotyledon, and *vice versa*. Examining the styles of many other species following foreign pollination, Strasburger noted that the phenomenon was of frequent occurrence and drew the important conclusion that pollen-tube growth could not be explained on the basis of sexual affinity. He suggested that, since pollen-tube growth in the style resembles so closely the penetration of the hyphae of parasitic plants into the tissues of their hosts, very probably a similar cause underlies both phenomena.

The early hybridists, notably Koelreuter (1761–1776) and Gärtner (1849), encountered a condition in certain plants whereby seed either failed to set or set incompletely after self-pollination, in spite of the fact that the pollen and egg cells themselves were normal as demonstrated by their ability to function completely in certain cross-pollinations. Among animals, Castle (1896) demonstrated that the hermaphroditic tunicate *Ciona intestinalis* exhibits a similar condition. This phenomenon, in which fertilization of the eggs by the male gametes of the same individual is impossible or at best irregular, is designated as *self-sterility*. It has recently been shown by East and Park (1917) that cross-sterility identical in nature with self-sterility occurs. While the number of cases in which self-sterility has actually been demonstrated is not large, East and Park contend with good reason that it is possibly a widespread phenomenon. Knuth (1906) has compiled a list of cases, the majority of them well founded, indicating that among the angiosperms the condition is rather generally distributed.

It was early shown by Scott (1865) and by Fritz Müller (1868) that pollen tubes are produced freely in the styles of self-sterile plants after selfing. Jost (1907) assigned as the immediate cause of self-sterility the failure of the pollen tubes after an incompatible pollination to grow rapidly enough to reach the ovary before the flower falls. Martin (1913), working with *Trifolium pratense*, a self-sterile form, found that the difference between self- and cross-pollinations was one of rate of growth. East and Park (1917), in their studies on Nicotiana, have secured abundant evidence to show that, following incompatible combinations, the rate of pollen-tube growth in the style is lower than that attending fertile combinations.

In that class of self-sterile plants designated as *heterostyled*, Darwin (1877) has shown that the peculiar fertility relations obtaining between the different forms of a given species are related to the differences in length of style and in size of pollen grains. In *Lythrum salicaria*, for example, each of the three forms, namely, the short-styled, the mid-styled, and the long-styled types, bears in its flowers two sets of stamens different in appearance and function. In this heterostyled species eighteen different pollinations are possible only six of which result in complete fertility. In a series of carefully executed experiments Darwin demonstrated that each form is sterile with pollen from its own two sets of stamens and fully fertile only with pollen from the set of stamens of corresponding length in each of the other two forms. That within the flowers of one and the same plant there are differentiated two kinds of pollen so strikingly unequal in their potency when combined with a plant of another form as to result in the production of a full complement of seed in the one case and to prove unfruitful in the other is indeed a phenomenon at once extraordinary and perplexing.

It is probable, however, that the immediate cause of unfruitfulness following illegitimate combinations among heterostyled species is not unlike that responsible in cases of ordinary self-sterility. Illegitimate matings are sometimes followed by the production of a few seeds that will produce normal plants. This fact, taken in conjunction with Darwin's observation that, following illegitimate pollination, pollen tubes are freely produced in the styles, supports the view that here too the impediment to fertility is entirely a physiological one.

It will be evident from the foregoing discussion that the general problem of pollen physiology is one of considerable biological interest. The simple mechanical methods by which pollen grains reach the stigma of the female sex apparatus have long been familiar facts. General agreement has been reached on the behavior of the male gamete in the presence of the egg itself. But as to the fundamental processes involved in the growth of the pollen tube with its cargo of nuclear material from stigma to ovules there is still wanting a comprehensive interpretation based upon a sufficient body of well ascertained facts.

The present studies were undertaken with the object in view of gathering some further evidence of the requirements for pollen-tube development and growth. The extent to which the known facts will permit of a general interpretation of the phenomenon will, we hope, be indicated in the succeeding pages.

THE CHEMICAL COMPOSITION OF POLLEN AND CONDUCTING TISSUE

Mangin (1889) distinguished two layers in the pollen-grain wall. The external cutinized layer he called the *exine;* the *intine* or internal layer was found to consist of cellulose on its outer side, associated with some pectic substance which he named *callose.* Biourge (1892) confirmed these observations and noted that early germination consists of an evagination of the intine.

Czapek (1913, 1920, 1921) has brought together the results of the earlier chemical analyses of pollen. The evidence these afford, taken in conjunction with that obtained through microchemical studies by Lidforss (1899 *b*), Molisch (1893), and other investigators, indicates that the pollen grains themselves, like the seed, are provided with a considerable amount of food material in the form of carbohydrates or fats. The nature of this reserve material in a given form, according to the views of Molisch (1893), Lidforss (1899 *b*), and Sterner (1913), is dependent to a certain extent upon age of pollen, seasonal conditions, and geographic distribution. Heyl (1917) observed three nuclei in the pollen grains of ragweed and estimated the nuclear volume to be about 20 percent of the protoplasmic substance. Chemical analyses of the entire grain showed 24.4 percent protein, 10.8 percent fat, 0.75 percent lecithin, 5.4 percent ash, 7.5 percent pentosans, 12.2 percent crude fiber, and small amounts of sucrose, glucose, and dextrin. On continuing his investigations, Heyl (1919) determined that the walls of the pollen grains in this form constitute 65 percent of the structure. The results of the detailed analysis Heyl has made of the protein extract of ragweed pollen are especially instructive. A considerable quantity of non-protein nitrogen was found. The aqueous extract contained 1.2–1.5 percent coagulable albumin and a mixture of proteoses amounting to 3.0 percent. Peptone was found, and the hexone bases arginine, histidine, and lysine were present in abundance, arginine being found in the smallest proportion. Only a minute quantity of organic phosphorus was present. Extracting with dilute alkalies, a glutelin amounting to 2.9 percent was obtained. Heyl and Hopkins (1920) have, on further investigation, found that the albumin present in ragweed pollen is a normal protein except that it lacks histidine and perhaps tryptophane. The percentage of amino-acids found by these authors in the ragweed-pollen proteins is as follows:

	Proteose 5/10–10/10*	Proteose Entire Fraction Dialysed	Glutelin	Albumin
Arginine	1.48	2.08	4.69 4.70	6.15
Histidine	0	0	1.69 2.30	0
Lysine	3.70	4.48	7.66 6.60	8.76
Tyrosine	.78	1.10	4.7	2.79 2.83
Tryptophane	0	0	0	0

* Saturation of $(NH_4)_2SO_4$.

Glutelin, which is the predominant protein, has histidine present among the bases and is therefore more nearly complete. The authors conclude that in pollen we have a series that may represent three stages of protein development: Native compound proteose (N = 5.4 percent) → albumin (-histidine) → glutelin. In the synthesis of protein in the growing pollen tube, Heyl and Hopkins have not considered the possibility that nitrogenous compounds are derived from the stylar tissue also. Anderson and Kulp (1922) have made extensive analyses of maize pollen. The principal reserve materials in this form appear to be carbohydrates in the forms largely of starch and sucrose. The percentages of starch and sucrose in three different varieties are reported as follows:

	Yellow Dent (Leaming)	White Flint (Luce's Favorite)	Pop Corn
Starch..................	11.07	19.04	18.03
Sucrose.................	9.09	2.97	14.18

The difference between the total amount of starch and sugar in the pop-corn pollen and that found in the other two varieties is large and perhaps of some significance. The differences in relative proportion of sugar and starch may not be important, since these are readily transformed one to the other. An analysis of the ash of Leaming pollen showed that potassium and phosphorus predominate, there being 35.58 percent of the former element present and 18.92 percent of the latter. Only 1.02 percent of calcium was found and 4.60 percent of magnesium. It was calculated that there is 3.62 percent of lecithin present in maize pollen. While the reserve food materials constantly found in pollen may be sufficient for initial growth, it is inconceivable that they are adequate in amount for the production of tubes that reach a length in certain species equal to many hundred times the diameter of the pollen grain itself. In the course of its development the pollen tube must draw upon outside sources of nutritive material.

The rôle of the conducting tissue in nourishing the pollen tubes was first defined by Amici (1824), the discoverer of these structures. Dalmer (1880) quotes Behrens (1875) as concluding from the results of his microchemical studies that the slimy substances of the stigma contain amyloid materials, sugar, and probably other carbohydrates. The anatomical investigations of Capus (1878) and of Dalmer (1880) revealed that reserve food materials are freely distributed in the tissues leading to the ovules. Green (1894) found that in the style of the lily the epithelial cells lining the stylar canal and several layers beneath them are filled with minute granules of starch. The outer layers of the style are free from these. Starch is plentiful, too, in the outer soft tissues of the fibrovascular bundles, indicating a definite deposit or reserve store placed in the conducting tissue. Green concludes, from the fact that the deposit does not extend to the stigma, that the reserve material in the style is intended for the growth of the pollen tube after it has exhausted the special store in the pollen grain. In Micrampelis, Kirkwood (1906) found that the cells lining the stylar canal and covering the placental lobes are filled with starch. A similar condition was observed in Melothria and Cyclanthera. The conducting tissue in *Cucurbita pepo*, however, appeared to be quite devoid of starch.

This does not necessarily indicate, as Kirkwood has observed, that the style of the pumpkin entirely lacks carbohydrates. Here, as in the other cucurbits examined, the style may provide nutritive material to the growing pollen tube in the form, probably, of cane sugar. Martin (1913) found that the exposed portions of the papillae of the stigma of *Trifolium pratense* are rather heavily cutinized. No starches or sugars were found, but an oily emulsion such as he had identified in the pollen is present.

Enzym Relations

Intimately related to the phenomenon of food-storage and utilization is the occurrence of enzyms in the pollen and conducting tissues. Erlenmeyer (1874) found evidence of diastatic activity in pine pollen. Van Tieghem (1886) observed that, following the addition of pollen of Narcissus, Crocus, and some other species to a 10 percent solution of cane sugar, with or without an antiseptic, the cane sugar was inverted. When pollen grains of some species were germinated in the presence of starch paste, Strasburger (1886) found that the starch was gradually hydrolyzed to sugar, indicating the presence of diastase. Green (1891), in a series of carefully conducted tests, demonstrated that diastase occurs in the pollen of Lilium, Helianthus, Antirrhinum, and several other species. In a second paper, Green (1894) reports the results of more extensive experiments. He found that diastase and invertase are frequently present in resting pollen but that their distribution is irregular, some forms containing both, others only one of them. Neither a cytolyst nor a proteolyst was found. Green's results on the changes in the amounts of enzym present during germination and growth are instructive. Lilium and Narcissus were selected as yielding diastase and invertase respectively. Quantitative determinations of enzymic activity were made with each form both upon fresh pollen and after cultivation on sugar solutions. A striking increase in reducing power was obtained with the pollen of Narcissus following germination. In Lilium it was found that, after several hours' growth, the diastatic power had increased as much as five times. Only a single exception to these results was observed. In a trial in which *Lilium pardalinum* was used, there appeared to be a temporary diminution in the quantity of diastase at the onset of germination. Lack of material prevented the repetition of this experiment. Working with a sample of Lilium pollen which had entirely lost its power to germinate through age, it was found that the amount of diastase present was only one third that originally contained in the fresh material.

Continuing his work, Green found that the pollen of *Zamia Skinneri*, one of the Cycads, contains neither starch nor diastase in its resting condition but that both were formed when the pollen was cultivated in solutions of sucrose or glucose. In water cultures alone, neither starch nor enzym was formed.

During the growth of the tubes of Lilium on artificial cultures, Green was enabled to observe directly the digestion of the starch carried over from the pollen grains. Shortly after germination the starch granules, previously staining blue with iodine, were observed to be changing color. As the tubes increased in length, the granules nearer the grain end still stained blue, while those studding the greater part of the tube were purple. Toward the tip of the tube the granules were distinctly reddish. As Green concluded, "the starch was evidently in process of digestion by the diastase, ministering to the great formation of cellulose composing the wall of the tube."

Rittinghaus (1887) observed that the pollen tubes of Lychnis, Agrostemma, Phlox, and some other forms in passing into the stigma exerted a solvent action on the cuticle. He suggested that this might be due to an enzym. Kamman (1904) found diastase, invertase, and a proteolytic enzym in rye pollen. In a later paper Kamman (1912) reports the presence of lipase also. Sandsten (1909) found diastase and invertase in the pollen and styles of a number of plants. In some species of Cassia, Herman Müller (1883) found two kinds of pollen which he termed *Befruchtungs-pollen* and *Beköstigungs-pollen* respectively. Only the former produces tubes under ordinary conditions. Tischler (1910) found that the "*Beköstigungs-pollen*" could be induced to germinate by adding saliva or diastase to the culture and attributed its sterility to a lack of this enzym.

Recently Paton (1921) has reported the results of extensive studies on the occurrence of enzyms in the pollen of 18 species of plants distributed among 14 genera in 9 families. Catalase, diastase, invertase, reductase, and pectinase were found in all; pepsin, trypsin, erepsin, and lipase were found in some but not in others; cytase was not demonstrated with certainty in any; zymase was doubtfully identified in apple pollen; tyrosinase and laccase appeared to be entirely absent. The wide occurrence of pectinase is interesting in view of the generally observed intercellular growth of pollen tubes in the style. The rôle of enzyms in the metabolism of the pollen tube is unquestionably of considerable significance. It is to be hoped that Paton's contribution on the occurrence of a variety of catalysts in pollen may lead to further investigation of the processes in which they are involved.

The Artificial Culture of Pollen

Since von Mohl (1834) observed that pollen of some species would form tubes readily in moist air, numerous attempts have been made to cultivate it artificially. It has been found that different species vary considerably in the ease with which their pollen may be germinated *in vitro.* In some cases tubes may be induced to form under a rather wide variety of conditions, in others the requirements for germination are quite exact, and with not a few species the methods thus far devised have given only negative results. Even in those cases in which germination of the pollen is possible, the growth subsequently obtained is, except in a few forms, almost negligible in amount when compared with that occurring normally in the style of the plant.

In spite of the varying degrees of success that have attended the attempts to cultivate pollen *in vitro*, the results that have been obtained with some forms show it to be a logical and promising method of attack upon the problems of pollen physiology. The seemingly inordinate variability sometimes encountered should not be permitted to obscure the results of experiments where regularity does prevail. The divergent results frequently obtained in artificial cultures of pollen are not, in the author's opinion, to be attributed entirely to the basic differences in the material that they might on first thought suggest, but rather to its somewhat capricious nature, resulting in fluctuations of a minor sort which an imperfectly developed technic fails to set forth in true perspective. Success depends in large degree upon the elimination of these more superficial variables by the choice of material in which they are at a minimum.

While the small amount of evidence on some phases of the problem might not recommend such a treatment, the extent of our knowledge of the requirements for pollen-tube growth and the directions in which further research might profitably be made will best be indicated perhaps by classifying the subject matter to permit of separate consideration of the carbon, oxygen, nitrogen, and mineral requirements, water, osmotic, and acidity relations, and the amount and kind of growth obtained.

Materials and Methods

During the course of these studies the extensive resources of plant material maintained in the Arnold Arboretum, the Harvard Botanic Garden, and at the Bussey Institution have been freely drawn upon. Of the many species tested, but a few were found which lent themselves readily to our purposes. Whenever possible the plants yielding suitable pollen were grown in the greenhouses at the Bussey Institution, and in so far as it could be readily done the supply of pollen was prolonged beyond the usual season. The pollen of Vinca, Scilla, Puschkinia, Chionodoxa, Muscari, and Lythrum used was largely gathered out of doors. Our inability to extend the flowering period of some very desirable forms proved to be a real handicap and is responsible to a certain extent for the incompleteness of some of the experiments reported.

Semi-solid media with agar as the basis were found the most suitable in cultivating pollen artificially. Except where otherwise mentioned, common shred agar and granulated cane sugar of the trade were employed. A petri dish in which a thin layer of agar is poured and allowed to set may be readily handled on the stage of a binocular microscope while the pollen is being plated. With the aid of a needle, a heap of grains transferred to the culture can be separated with facility and arranged in regular lines.

When thus oriented, subsequent examinations may be made systematically without danger of confusion. Such an arrangement is especially convenient when it is necessary to make repeated measurements on each of a series of tubes, as in securing data for growth curves.

Except in a few of the early experiments in which a lower power was used, the pollen tubes were measured with a 7.5 ocular micrometer used in conjunction with a 16-mm. objective. Where the pollen tubes growing on artificial media take a rather straight course or do not change their direction frequently, their lengths may be measured to within 40 μ. Where there is considerable deviation from a straight line, the error is, of course, larger; but among such forms as Cucumis, Scilla, and Vinca, such tubes are not very frequent. With practice even these can be measured with a fair degree of accuracy. Whenever possible, average values have been obtained based on the measurement of from 50 to 125 tubes. When the tests are repeated also, this procedure overcomes to a considerable extent, we believe, the inherent shortcomings of the material for such quantitative experiments as we have made. In obtaining points for growth curves it was found necessary to make frequent, and, if a number of tubes were involved, rather rapid, measurements during the early stages of elongation. Very fortunately, pollen tubes of Scilla, Vinca, and Cucumis during this period maintain a fairly straight course, and their lengths may be readily determined to within 25 μ. As a tube grows longer its direction may be rather frequently altered, but in the forms mentioned, in marked contrast to the vagrant tubes of Hippeastrum, for example, these digressions do not present serious difficulties.

The cultures were usually grown at room temperature and were set away in a drawer or covered on the table. These latter precautions were perhaps unnecessary, since the diffused light of the laboratory was apparently without influence on growth.

Carbon Requirements

It is a fundamental and familiar fact that, in the formation of the organic material of the green plant, CO_2 serves as the primary source of carbon. It is the chloroplasts alone, and these only when they contain chlorophyll, that are active in its assimilation. Plants devoid of chlorophyll, or particular organs lacking it, are wholly unable to utilize the carbon supply of the atmosphere. That the formation, however, of the widely distributed reserve food material, starch, does not necessarily stand in direct connection with this assimilation of CO_2 is shown by the fact that green leaves floated upon sugar solution of suitable concentrations in the dark will form starch. Even in the green plant, then, sugar may be interposed as a source of carbon. It is commonly accepted that in plants lacking chlorophyll, such as the fungi, sugars obtained *ab extra* regularly serve to fill the carbon requirements. Within the body of the green plant, the cells remote from the chlorophyll tissues derive their supplies of carbon from the sugars moved from the green parts. No chlorophyll is present in the pollen tube, of course, and beyond the reserves in the pollen grain it likewise is dependent upon outer sources for its carbon supply. There is good presumptive evidence that it derives these directly from the stylar tissues in the form of readily diffusible sugars. The results of studies on pollen-tube nutrition *in vitro* throw considerable light on this phase of the problem. Since Schleiden (1849) and Van Tieghem (1869) made the first serious attempts to cultivate pollen artificially, cane-sugar solutions have been frequently employed as media and with some success. Of the numerous studies that have been reported, however, a few only contribute evidence of critical value in determining the part played by sugar as a nutrient. The reserve materials of the pollen grains themselves are probably sufficient to support an appreciable amount of growth, and it must be considered that the sugars added might by a purely osmotic action so adjust the physical conditions of the medium that growth could proceed until this stored material was exhausted. In pollen cultures where the amount of growth obtained is meagre—and these unfortunately comprise most of the reported cases—it is impossible, therefore, to define with certainty the action of a substance incorporated in them. If, however, on the addition of material of nutritive value, growth results clearly in excess of the amount that the reserve materials of the pollen grains could possibly support under the most favorable physical conditions, it would be justifiable to conclude that the added substance had actually been assimilated and had contributed to the formation of the pollen tube. A few examples of this sort are to be noted.

Jost (1907) secured pollen tubes of *Hippeastrum aulicum* 17–22 mm. in length on agar cultures containing 1 percent of cane sugar. When 1/4–1/2 percent of glucose was substituted for the cane sugar, the tubes attained a length of 7–8 mm. Knight (1917) reports that Miss Eckerson secured some pollen tubes of apple 10 mm. in length in 3-percent solutions of fructose. Bobilioff-Preisser (1917) found that the pollen of *Vinca minor* in 1–10 percent cane-sugar solutions formed tubes 10 mm. in length. The writer, working with pollen from an Hippeastrum plant of hybrid origin, has secured numerous tubes over 10 mm. in length on 2-percent agar cultures containing 3 percent of cane sugar. Very commonly two tubes are emitted from a single pollen grain in this form the total length of which frequently exceeds 10 mm. Pollen of *Vinca minor* cultured on 2-percent agar with 5 percent of sugar added produced tubes 7 mm. long. In *Nicotiana alata* some tubes reached a length of over 100 times the diameter of the pollen grain on agar plates containing 15 percent cane sugar, and in Scilla, in which the pollen grain measures about 50 μ through its longest axis, tubes were secured, on agar cultures to which only cane sugar had been added, 4 mm. in length or about 80 times as long as the pollen grain itself.

While we have no exact quantitative evidence in these cases to show that the amount of dry matter has actually been increased during growth, microscopic observation strongly favors such a conclusion. Tubes are formed with cellulose or callose walls reaching a length in some cases equal to several hundred diameters of the pollen grain and containing in their frequently bulbous tips a considerably enhanced mass of protoplasm. These tubes, moreover, commonly form numerous callose plugs the total volume of which may alone exceed that of the grain. The inference is clear that the sugars in the medium have been drawn upon in the development of these structures. It would appear also that sucrose, glucose, or fructose may serve as sources of carbon although all are not equally suitable in a given case.

There is another line of evidence which also points to the conclusion that sugars are taken up by pollen tubes. Mangin (1886) first noted that pollen grains of some species when placed on sugar cultures deposited starch freely. Tischler (1917) has confirmed this observation. Dodel-Port (1880) also found that pollen of *Pinus Laricio* on concentrated sugar solutions formed numerous large amylum grains. Green (1894) observed that the fresh pollen of Zamia contains no starch but that it appeared regularly in the grains when cultured with sugar. While these facts show that pollen grains may take up sugar and deposit starch initially, and suggest that the process of sugar-absorption may be maintained throughout the growth of the tube, we should not expect, for reasons to be considered later, that the deposition of starch would continue during growth under artificial conditions. Such a deposition is not, of course, a necessary consequence of sugar-absorption, and failure to detect starch in growing tubes would not vitiate the conclusion that sugars from without were being utilized.

OXYGEN REQUIREMENTS

Van Tieghem (1869) first demonstrated the necessity of oxygen for germination. He sowed pollen in a drop of water, placed a cover glass over it, and noted that only the grains at the periphery emitted tubes. When the cover glass was lifted, the remainder grew. Van Tieghem also secured quantitative evidence showing that during the elongation of the pollen tube, accompanied by the digestion of the starch within it, oxygen was removed from the surrounding air and replaced by carbon dioxid. Mangin (1886) reported that on artificial cultures the respiratory ratio falls gradually from the time of germination, and that, moreover, the quantities of gas taken up or given off decrease gradually until the cell dies. Pollen lacking starch did not readily germinate in media deprived of sugar, and it was found that the respiratory ratio under such conditions was four to five times less than in sugar solutions. While Mangin does not state the nature of the reserve material in these cases, it is probable that in some of them, at least, unsaturated fats were present, the digestion of which would give the low respiratory ratios observed. The marked acceleration of the respiratory processes in the sugar media suggests a direct utilization of this carbohydrate.

Reprinted from the AMERICAN JOURNAL OF BOTANY, XI: 283-294, May, 1924.

THE PHYSIOLOGY OF POLLEN

II. FURTHER CONSIDERATIONS REGARDING THE REQUIREMENTS FOR GROWTH[1]

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(Received for publication July 5, 1923)

NITROGEN-ASSIMILATION AND THE POSSIBLE RÔLE OF GROWTH-ACCESSORY FACTORS

Tokugawa (1914)[2] noted that the addition of some protein materials to pollen cultures often more than doubled the growth. Knight (1917), coöperating with Miss Eckerson, reports that, with the germination and growth of the pollen tubes of apple, asparagin makes its appearance. Miss Eckerson found, moreover, that the addition of traces of asparagin to artificial cultures of apple pollen accelerates the rate and increases the total amount of pollen-tube growth.

The writer has made numerous tests of the effect of the acid amide asparagin on pollen-tube growth without, however, observing any case in which its addition to the cane-sugar medium increased growth. Pollen of Nicotiana, Cucumis, Scilla, Vinca, and several species of Pyrus was tried on cultures in which the concentration of asparagin ranged from 1/2000 to 1 percent. At the higher concentrations growth was markedly retarded. The amino acid glycocoll similarly failed to give positive results. Some evidence was secured, however, to show that the addition of traces of peptone promotes growth. Inorganic forms of nitrogen, as KNO_3 and $(NH_4)_2SO_4$, gave negative results. Growth was checked by these salts even when present in extremely small amounts.

A very considerable improvement in the artificial cultures was secured, however, through the incorporation of sterile yeast. This was prepared by working up a cake of Fleischmann's compressed yeast with 100 cc. of water and boiling the suspension 1 to 2 minutes. Because of the tapioca flour in the commercial article, the resulting preparation is thick and viscid. If the flour is separated by sedimentation before boiling, a clear yellowish extract of the yeast can be prepared which still retains the growth-promoting properties of the cruder material. This latter, however, is more readily prepared and was used in subsequent tests.

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[1] Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

[2] The references are to literature cited at the end of the fourth paper of this series, to appear in a future issue of this JOURNAL.

TABLE 1. *The Effect on the Growth of Pollen Tubes of Cucumis sativus of Adding Live Yeast, Sterile Compressed Yeast, and Sterile Brewer's Yeast to Sugar-agar Culture Medium (Lengths Given in Microns)*

Test	Check		Sterile Compressed Yeast		Sterile Brewer's Yeast		Live Yeast	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1..........	99	422	100	571	81	420	—	—
2..........	58	600	63	626	69	579	65	324
Average....	157	488	163	592	150	494	65	324

A sample of brewer's yeast was found unsatisfactory because of impurities. The material obtained was taken from the bottom of a vat and contained a considerable amount of sediment difficult to remove. Living yeast, added to the sugar medium, gave rise to alcoholic fermentation which checked growth. The results of comparative tests between compressed yeast and the sample of brewer's yeast mentioned above, both prepared by boiling, and living yeast are given in table 1.

TABLE 2. *Influence on the Growth of Pollen Tubes of the Addition of Sterile Yeast to Culture Media (Lengths Given in Microns)*

Test No.	Test Species	Concentration Sugar (percent)	Without Yeast		With Sterile Yeast, 2 drops per 25 cc.		
			No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	Percent Increase
1.	*Cucumis sativus*	10	111	573	91	1270	121
2.	*Cucumis sativus*	10	43	753	71	932	24
3.	*Cucumis sativus*	10	74	1710	78	758	−55
4.	*Cucumis sativus*	10	69	261	59	707	170
5.	*Primula obconica* (long-styled) ..	10	64	175	101	310	77
6.	*Primula obconica* (long-styled) ..	10	106	171	109	341	101
7.	*Primula obconica* (short-styled) .	10	96	326	100	496	53
8.	*Primula obconica* (short-styled) .	10	107	365	114	551	51
9.	*Primula obconica* (long-styled) ..	10	86	67	114	124	85
10.	*Primula obconica* (short-styled) .	10	111	133	111	218	65
11.	*Lythrum salicaria* (green pollen).	15	26	379	40	1503	296
12.	*Lythrum salicaria* (green pollen).	15	34	671	40	863	28
13.	*Lythrum salicaria* (green pollen).	15	69	590	89	1279	117
14.	*Lythrum salicaria* (yellow pollen)	15	48	277	53	1120	304
15.	*Lythrum salicaria* (yellow pollen)	15	47	473	58	532	13
16.	*Lythrum salicaria* (yellow pollen)	15	60	439	55	840	92

Table 2 gives the results obtained in 16 tests with pollen of three different species. The boiled yeast was added to the appropriate cultures at the rate of two drops per 25 cc. of the sugar-agar medium. When pollen of Cucumis and Primula was used, germination was almost perfect throughout. With Lythrum, on the other hand, the percentage of grains germinating was lower and variable, though independent apparently of the yeast in the medium, as table 3 shows.

Table 3. *Percentage Germination of Pollen of Lythrum salicaria on Media with and without Sterile Yeast*

Test	Percent Germination	
	With Yeast	Without Yeast
11	48.7	37.7
12	54.8	53.1
13	89.9	85.2
14	75.7	49.0
15	79.4	76.3
16	57.9	69.8

The increases in growth on the sterile yeast media are for the most part very considerable. In test 3 alone, in which cucumber pollen was used, the plain sugar culture gave a greater average growth. In the other three tests in which pollen from the same form was used, the increases with the addition of yeast were 24, 121, and 170 percent respectively. It was early found that with Cucumis extreme care was necessary to get uniform samples of pollen. The results of trials made on a constant medium justified the precaution that was taken in the experiments of securing material for a given test not only from one flower but from a single anther within the flower. Of the causes contributing to the variability in the behavior of pollen from different anthers of the same flower, nothing is known. Differences in exposure to light may be responsible, although we have no evidence to show that such is the case.

During the course of experiments with other kinds of pollen, the results of which are not included in table 2, it was quite obvious that when the yeast preparation was added growth was materially greater than on media lacking it.

There is an increasing amount of evidence that the addition of extracts of fresh plant tissues of various sorts stimulates the growth of pollen tubes on artificial cultures. East and Park (1918) obtained some evidence to show that stigmatic extracts of *Nicotiana angustifolia* and *N. forgetiana* promoted the growth of pollen tubes of Nicotiana *in vitro*. Knowlton (1922) observed that the percentage of germination of Antirrhinum pollen was greatly increased by the addition of minute amounts of crushed stigma to cultures of the same species. Pollen of this form which had been stored in oxygen at 10°–22° C. for 670 days failed to grow in sugar solutions, but, on the addition of a piece of stigma, 10 percent germination was secured. Pollen kept in air for the same length of time, similarly would not germinate in sugar solutions, but gave 50 percent germination on the addition of stigmatic tissue. Maize pollen, which can be germinated *in vitro* only with difficulty, Knowlton found did not respond to the addition of stigmatic parts or decoctions of the stigma. Tokugawa (1914) obtained similar results when crushed stigmatic tissues were incorporated in pollen cultures of a number of species not readily grown *in vitro*. It is quite probable that in these cases the media were unadjusted in some other important respect for the growth of the particular species used. The important point is that, with forms which do lend themselves to present artificial culture methods, growth is appreciably enhanced by the addition of some fresh plant tissues.

While the present writer's work on this point is not extensive, some further interesting facts have been secured. It has been observed that the crushed stigmas of Nicotiana added to agar cultures containing 15 percent cane sugar regularly increase the growth, not only of the pollen tubes of Nicotiana, but also of those of other forms growing at this concentration of sugar. Crushed ovarian tissue gave similar results. Striking increases in growth were secured on the addition of 1 drop of extract of raw potato to 25 cc. of sugar-agar medium. The average length of the pollen tubes of Cucumis was thus increased 43 percent. Larger amounts of potato extract tend to check growth. Tomato extract, which is very acid, retarded growth except in high dilution.

To what must we attribute the growth-promoting power of these sterile yeast and fresh plant materials? The evidence at hand does not permit a satisfactory answer. The fact that such preparations are complex leaves us in the dark as to the chemical nature of the active substances, and even precludes at this stage a definite conclusion that the gynoecium and potato extracts and the yeast preparation fall in the same category as regards their growth-promoting properties.

Some further evidence is available, however, on the nature of the material contributed by the yeast. In table 4 the results of experiments to determine the heat stability of its growth-promoting substances are summarized.

TABLE 4. *Influence on the Growth of Pollen of Cucumis sativus of Adding to the Culture Medium Yeast Boiled 1 Minute, 15 Minutes, and Autoclaved for 45 Minutes at 120°C. (Measurements Given in Microns)*

Test	Check without Yeast		With Yeast Boiled 1 Minute		With Yeast Boiled 15 Minutes		With Yeast Autoclaved for 45 Min. at 120° C.	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1.......	167	494	172	571	157	608	—	—
2.......	97	647	129	1369	—	—	144	1255
3.......	109	463	114	661	—	—	115	534
4.......	123	506	110	702	—	—	115	612

In these tests Cucumis pollen was used. A check culture consisting of 2 percent agar and 10 percent cane sugar was made for comparison. In the other cultures, yeast prepared as indicated in the table was added at the rate of 2 drops per 25 cc. of medium. Boiling for 15 minutes does not result in any diminution of the growth-promoting power of the yeast. Autoclaving for 45 minutes at 120° C. diminishes its potency somewhat, but the material still retains its original properties to a marked degree.

It would not be surprising to find that the expressed juice of the other plant tissues mentioned reacted similarly to heat. The high peroxidase content of the potato may enhance its value somewhat, but the similarity of the growth-promoting powers of all the materials mentioned above suggests that some constituent common to all is responsible. It is not improbable, though, that the growing tubes utilize more than a single constituent of the sterile yeast. Whatever these substances may be, they are effective in small amounts. Tests showed that the yeast, as would be expected, loses its power to invert cane sugar in the boiling. Presumably all the enzyms present in the living cell are thus destroyed. Since an excess of carbohydrates in the form of cane sugar was already present in the culture, it is unlikely that the yeast contributes further in this regard. As will be seen later, the mineral constituents may account for a small part of the increased growth obtained. What of the remainder? Perhaps some water-soluble, heat-stable nitrogenous compound, a protein derivative, is responsible. Or possibly the growth-accessory substances, whatever their chemical nature may be, that Thjötta and Avery (1921) have found in plant tissues promoting the growth of hemophilic bacilli are the active substances. Dutcher (1918) has demonstrated the presence of the vitamin water-soluble B in maize pollen. Nothing is known, however, of the significance of such substances in pollen-tube metabolism.

Osmotic Relations

A source of no little annoyance and perplexity to those who have attempted to cultivate pollen artificially has been the bursting of pollen and pollen tubes. No satisfactory explanation of this phenomenon has as yet been brought forward. It has been noted repeatedly that the amount of bursting bears no clear-cut relation to the concentration of sugar in the culture. Van Tieghem (1869), Correns (1889), Molisch (1893), and Lidforss (1896) independently have concluded from this fact that the amount of bursting is not related to the osmotic force of the surrounding medium. With this conclusion we can not agree. While additional factors associated with the colloidal nature of the protoplasm may perhaps be involved, the evidence favors the view that it is largely an osmotic phenomenon. The conclusion that it is not an unbalanced osmotic condition that is the cause of bursting has been based on the assumption that the protoplasmic surface of the pollen grain acts as a semipermeable membrane to sugar solutions. There is no good evidence to show that this, in general, is the case. Since, from the standpoint of culture methods, the matter is of prime importance, and is moreover of considerable theoretical interest, we will review the evidence relating to it in some detail.

That pollen tubes of many species will grow readily on sugar solutions varying widely in concentration is attested by the results of numerous investigations. Molisch (1893) pointed out that the optimum concentration of sugar for germination varies widely. *Lilium Martagon* germinated in solutions containing from 1 to 50 percent sugar, and *Deutzia scabra* in 1- to 40-percent solutions. Pollen of some other species germinated over a much narrower range. Molisch also calls attention to the fact that the behavior of those species which germinate at high concentrations finds a parallel in the results given by certain fungi. Tokugawa (1914) states that the pollen grains of each species possess a definite turgor, and that for growth it is necessary that conditions be accommodated to this. But Tokugawa's data will not support the view that the range of *sugar* concentrations over which pollen grains will germinate is narrow, or that in many cases at least there is even a well defined optimum. He found, for example, that pollen of *Licorice aurea* grew in cane-sugar solutions ranging from 0.1 M to 1.8 M, and that there was little difference in the results between the concentrations 0.6 M and 1.0 M. Similar results were secured with other species. Adams (1916) secured germination of apple pollen on media containing from 5 to 30 percent cane sugar. Nicotiana pollen, as East and Park (1918) have shown, will grow on agar media containing from 0 to 30 percent sugar. Tischler (1917) found that pollen of *Plantago media* gave good germination in sugar solutions up to 50 percent. Knowlton (1922) observes that Antirrhinum pollen germinates best at one time in one concentration of sugar and at another time at another concentration. Our results with a number of other species are in substantial agreement with this finding. It has been our experience also that often pollen from the same anther will give quite similar results when grown on media containing very different amounts of cane sugar. In table 5 are given the results of a typical experiment with Cucumis pollen.

TABLE 5. *Results of an Experiment to Determine the Effect of Concentration of Cane Sugar on Total Amount of Growth of Cucumis Pollen Tubes (Measurements Given in Microns)*

	Conc. Sugar (percent)			
	10	15	20	25
No. of tubes measured	93	92	132	115
Average length	147	199	214	179

The very considerable body of evidence, showing that pollen grains will germinate and produce strong tubes in sugar solutions of widely different concentrations, suggests the conclusion that the cell membranes of the pollen grain and its tube are or become permeable to cane sugar, and that the final result as far as osmotic pressure is concerned is the same as though the surrounding medium were water. How else may we interpret the facts? Perhaps osmotically active substances are produced within the cell, bringing it into equilibrium with the particular concentrations of sugar without. If this were so, we should expect a well defined optimum concentration of sugar for early growth, above which the pollen would plasmolyze and below which, if the wall could not sustain the pressure, the tubes would burst. But we find that Cucumis pollen for example will germinate almost *immediately* in each case when placed on media in which the concentration of sugar varies from 5 to 20 percent. Even at 25 percent there is no clear evidence of plasmolysis, and, while germination is somewhat slower, sufficient time does not elapse before the tube appears to encourage the view that in the interval much material of osmotic value could have been produced within the cell. We know of no mechanism inside the cell capable of such wide and rapid adjustment of sap-concentration as such an explanation would demand.

The fact that starch sometimes accumulates in pollen growing in sugar solutions is unmistakable evidence that sugar passes in, although little knowledge of the rate of diffusion is afforded thereby. Mangin (1886) noted that pollen tubes of some plants increase the size of their starch grains on sugar media. Mangin's observation has been confirmed by Tischler (1917), who, further, quotes Dodel (1878) as having found that Pinus pollen in concentrated sugar solutions formed many large amylum grains. Strasburger (1872) found much greater amounts of starch in the pollen tubes of *Pinus sylvestris* growing in stylar tissue than the contents of the original pollen grain could account for.

While we have found such experiments rather unsatisfactory, it does appear that, when pollen tubes are transferred from a culture of lower sugar concentration to one much higher, very little or no plasmolysis occurs. Some tubes of *Vinca minor*, grown in a 10-percent sugar culture with agar and transferred with a needle to a 50-percent solution of sugar, plasmolyzed throughout the greater part of their length only to regain their normal condition in a very few minutes. Such recovery is taken as evidence of the penetration of the sugar. Other tubes in the same transfer showed no plasmolysis. It would appear that in such cases the sugar penetrates so rapidly that no water is withdrawn from the tubes and shrinkage does not result.

Lloyd (1918) is inclined to regard bursting as due entirely to imbibition by the protoplast beyond the strength of the wall to confine it. This explanation leaves out of consideration the osmotic action of the dissolved materials in the tube and is vitiated, it seems to us, by certain facts regarding the amount of bursting that takes place when tubes grown on sugar solutions of different strengths are transferred to pure water. Lidforss (1896) placed pollen of *Lobelia cardinalis* for one hour on culture media containing 3, 2, and 1 percent of sugar respectively. On transferring the grains to distilled water, a large number of the first lot burst and a considerable number of the second; of those grown in 1 percent sugar, none burst. On Lloyd's hypothesis we are at a loss to explain these facts. On the other hand, if the pollen is somewhat permeable to cane sugar, so that, during the hour on the culture media, the original osmotic pressure of the solution within the tubes becomes increased by an amount approaching that of the surrounding solution, the tubes, when transferred to distilled water, will tend to burst the more freely the higher the concentration of the solution in which they were grown.

The evidence which Lidforss (1896) and other investigators have brought forward, showing that pollen of many species will germinate well in distilled water, indicates that the osmotic pressure developed in these cells is relatively low. Their turgor is largely due perhaps to electrolytes dissolved in the cell sap, to which the plasma membrane is less permeable than to sugars. While Martin (1913) found that the pollen grains of *Trifolium pratense* are permeable to saturated solutions of KNO_3 and NaCl, we should not attach much significance to such facts, since, as the researches of Osterhout (1922) and others have shown, such materials in unbalanced solutions may cause marked changes in permeability through injury. Initially, the surface of the pollen grain may in some forms act as a semipermeable membrane to sugar solutions, for, as Anthony and Harlan (1920) found, barley pollen is plasmolyzed in high concentrations and in low ones it bursts.

The writer has found that, while bursting of *pollen grains* of Cucumis is little in evidence on agar media containing various concentrations of sugar, the *tubes* burst freely during their rapid elongation; or they may remain intact up to one hour after their maximum length has been reached. The simplest explanation of these facts is that pollen is fairly permeable to cane sugar and that the pollen tubes may become more so during their growth. As the sugar diffuses in, to the osmotic force of the original cell sap is added that of the solution without, and bursting results whenever the pressure exceeds the resistance of the pollen-tube wall. The permeability of the protoplasmic membrane in various kinds of pollen may be different, and in a given case may be dependent to a certain degree upon growth conditions.

The Effects of Salts, and Some Remarks on Acidity

It has been found again and again that the addition of electrolytes to pollen cultures hinders growth or inhibits it entirely. Lidforss (1896) observed that NaCl, KNO_3, and $Ca(NO_3)_2$, even in small amounts, are toxic. Different species, however, reacted somewhat differently to these salts. $Ca(NO_3)_2$ was very poisonous for Nicotiana but relatively harmless to Lobelia pollen. KNO_3, on the other hand, injured Nicotiana pollen less than that of Lobelia. In a further paper, Lidforss (1899 *a*) reports that, while fresh albumin was toxic, pollen tubes reacted chemotropically to it after dialysis. Tokugawa (1914) found KNO_3, K_3PO_4, $CaSO_4$, $FeSO_4$, and $ZnSO_4$ injurious in various degrees to pollen from a number of different species.

The writer has observed that the addition to sugar-agar cultures of even small amounts of $NaNO_3$, $NaCl$, KNO_3, $CaSO_4$, $CaCl_2$, KH_2PO_4, and Na_2HPO_4 reduces the growth to a marked extent. The salts present in the boiled yeast preparation used in small amounts in our culture media do not retard growth, however. To test this point, a known quantity of yeast was incinerated over an open flame to a gray ash. This was taken up with water, and the salts were brought to the same concentration as in the sterile yeast preparation used. Cultures were prepared containing 1 and 2 drops of this solution per 25 cc., the latter being the concentration in which the whole yeast was being used, and the amount of growth of Cucumis pollen was determined on each of these media and on a check. The results are given in table 6.

TABLE 6. *The Effect of Yeast Ash on the Growth of Cucumis Pollen Tubes (Measurements Given in Microns)*

Test	Check		1 Drop Yeast-ash Solution		2 Drops Yeast-ash Solution	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1...	94	292	98	306	97	363
2...	84	365	73	310	80	483

We may reasonably assume that any toxic properties of the inorganic constituents of yeast would be manifested in greater degree when these exist as free salts than when they are partially combined with the protein complex, as is probably the case in the boiled yeast used. Our experiments indicate that in the free state yeast salts may promote pollen-tube growth to a slight extent; at least at the concentrations used they do not retard it.

Some experiments were made to determine the effect of sea water on pollen-tube growth. A series of cultures were prepared containing 2 percent agar and 10 percent cane sugar with a little sterile yeast, in which the distilled water ordinarily used was replaced with sea water to the extent of 6, 12, and 25 percent respectively. In table 7 the percentage of germina-

TABLE 7. *The Effect of Sea Water on Germination and Growth of Cucumis Pollen (Lengths Given in Microns)*

	Conc. of Sea Water			
	Check	6 percent	12 percent	25 percent
Percentage germination...............	97.7	98.1	70.0	3.8
Average length of tubes..............	561	549	284	112

tion and the amounts of growth of Cucumis pollen on these media and on a check without any sea water are given.

According to Dittmar's data as recalculated by Henderson (1913), the amounts of sea water here used represent concentrations of marine salts of 0.20, 0.41, and 0.85 percent. Less than 0.5 percent of sea salts depresses germination and growth markedly. In the 0.85 percent culture, but 2 grains in a total of 52 germinated, and these produced only short tubes. The injurious effects of sea water are very striking.

Interesting differences in the amount of pollen-tube growth were also found when the shred agar ordinarily used in the media was replaced by a specially prepared product, "Bactoagar." Analyses by Fellers (1916) show that this latter is somewhat lower in content of moisture, nitrogen, crude fiber, and ash than the common shred agar. Smaller but probably significant differences resulted, too, when chemically pure sucrose was used instead of the granulated cane sugar of the trade. In the experiments with these materials, the four possible combinations of agar and sugar were made and the percentage of germination and the amount of growth of Cucumis pollen were determined on each medium. The results are summarized in table 8, the average length of the tubes on the shred agar-commercial cane sugar medium in each test being taken as 100.

TABLE 8. *Summary of Results on the Effect of "Bactoagar" and C. P. Cane Sugar on Growth of Cucumis Pollen*

Culture Medium	Percentage of Germination (Average of 2 Tests)	Amount of Growth	
		Test 1	Test 2
Shred agar-com. sugar	87.5	100.0	100.0
Shred agar-c.p. sugar	70.9	84.8	86.1
Bactoagar-com. sugar	54.3	13.1	18.0
Bactoagar-c.p. sugar	64.1	13.7	23.8

In these two tests, 750 pollen tubes were measured. Since the respective values obtained in each experiment are of the same order, we are justified in attaching some significance to the results.

As a difference in pH was found between the two brands of agar used in the foregoing experiment, we should, before proceeding to an analysis of the results, consider the evidence, meager as it is, regarding acidity and alkalinity in relation to pollen-tube growth. Molisch (1893) observed that the stigmatic secretions of many plants are acid, turning blue litmus a deep red. He found, moreover, that pollen of *Azalea indica*, *Rhododendron ponticum*, and *R. arboreum*, which otherwise would germinate only on the pistils, grew beautifully in distilled water containing 0.01 percent malic acid or a little calcium malate. Citric acid failed to promote germination. Jost (1907) secured pollen tubes of *Rhododendron* sp. 12 mm. long on a medium containing 5 percent cane sugar and 0.01 percent citric acid, and noted further that the pollen was very sensitive to changes in the concentration of the acid. Strong acids and bases beyond slight amounts totally inhibited growth. Electrolytes added to a culture medium may influence growth through the chemical effect of their ions and molecules or by altering the reaction. From the small amount of evidence which Molisch and Jost have obtained, we cannot definitely assign the favorable effects of the acids used to either category. If, as it appears with Rhododendron, both malic and citric acid promote growth, their effect may reasonably be considered as due to a favorable adjustment of the reaction.

Our experiments to determine the effect of changes in pH on pollen-tube growth were unsuccessful. Very little or no growth could be obtained in the presence of acids and bases used, and the trials were abandoned. The culture medium used in the majority of the writer's other experiments, consisting of shred agar, distilled water, commercial cane sugar, and a small amount of sterile yeast, gave a reaction of about pH 6.6. When the shred agar was replaced by "Bactoagar," it was found that the acidity was considerably increased. Standards were not at hand for making determinations below pH 6.0, but the "Bactoagar" medium was at least as acid as this. A sample of gelatin which also failed to promote good growth was found to have an acidity of pH 6.0 or less.

As shown in table 8, the culture media containing "Bactoagar" gave lower germination and less than one fifth the amount of growth that was obtained when shred agar was used. According to Fellers (1916), "Bactoagar" is somewhat lower in ash content than the cruder product. Nothing is given, however, regarding the composition or proportions of the mineral constituents. Possibly these remaining salts are responsible for the toxic effect of the "Bactoagar," but on the basis of the facts at hand we are inclined to attribute its failure to promote growth to the high acidity found.

The most interesting fact revealed in our experiments was that cultures containing shred agar gave about 17 percent more growth with commercial cane sugar than with chemically pure sugar. This fact may be significant; at least it suggests a method of attack on the problem of the toxicity of salts to pollen tubes that has proved fruitful in studying their rôle in the physiological processes of various other forms. According to Fellers (1916), ordinary shred agar contains about 3.33 percent ash; the cane sugar of the trade contains slight amounts of mineral impurities. On the addition of chemically pure sucrose, shred-agar cultures give, as shown in table 8, an amount of growth equal to 85; when commercial cane sugar *with its mineral impurities* is substituted for chemically pure sugar, the amount of growth is increased to 100. The content of mineral material is undoubtedly variable in both agar and commercial sugar, though probably seldom over 0.5 percent in the latter. We are in the dark as to the chemical nature fo these salts; hence any conclusion as to their mutual effect must be tentative only. But the facts as far as they go indicate antagonism; in other words, the agar contains substances whose injurious effects as revealed by decreased growth when used with pure sucrose are diminished by the mineral impurities in the commercial cane sugar. Whether or not such is actually the case is a question that must await the results of more detailed investigation, but the suggestion is put forward at the present time as a working hypothesis.

We are in urgent need of further knowledge of the effects of salts on pollen-tube growth. As the extensive researches of Loeb (1906, 1912), Osterhout (1922), and others have shown, these play a cardinal rôle in determining the permeability of the cell membrane. If the technic of pollen culture *in vitro* can be so adapted as to permit a study of some of the conditions influencing permeability of the protoplasmic membranes of pollen and pollen tubes, much additional light may be thrown upon the problems of toxicity of salts, bursting, and the growth processes in general. Indeed, it is not entirely improbable that the slight amount of growth secured with many forms on our present artificial media is due to exosmosis of essential salts normally retained within the pollen tube.

Reprinted from the American Journal of Botany, XI: 351–364, June, 1924.

THE PHYSIOLOGY OF POLLEN
III. GROWTH *IN VITRO* AND *IN VIVO*[1]

R. A. Brink

(Received for publication July 5, 1923)

The Length of Pollen Tubes Grown on Artificial Media as Compared with the Distance from Receptive Surface to Ovary in the Plant

It was stated in an earlier paper in this series that the amount of growth of pollen tubes grown *in vitro* was, except in a few forms, almost negligible in amount when compared with the distance the tube must travel in the plant in order to effect fertilization. This fact has been emphasized by numerous workers, some of whom have called in question the value of artificial cultural studies on this account. It is a fact that even successful germination has been found difficult with pollen of many forms, and the subsequent growth in others that germinate freely is very feeble and irregular. Much of the variability in the behavior of pollen from forms that will from time to time give very satisfactory results remains to be accounted for. But the problems are not unapproachable; the insight already gained into some phases is no cause for apprehension.

An encouraging feature of the studies on methods of culturing pollen artificially has been the discovery of a few species that will produce tubes *in vitro* comparable in length to those growing on the style of the plant. Bobilioff-Preisser (1917),[2] working with *Vinca minor*, a form in which the styles are 0.5 to 0.8 cm. in length, secured pollen tubes about 1.0 cm. long on 1.5-percent agar media containing from 5 to 10 percent cane sugar. The writer has worked rather extensively with *Vinca minor* pollen and has secured some tubes as long as the style on sugar-agar cultures containing a little sterile yeast. Knight (1917) reports that about 5 percent of the pollen tubes of apple growing in a 3-percent fructose solution containing

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[1] Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

[2] References are to literature cited at the end of Part IV, to appear in a future issue of this Journal.

[The Journal for May (11: 283–350) was issued May 17, 1924.]

traces of asparagin attained a length of 10 mm., this being more than the required length to reach from stigma to egg. The writer has attempted to repeat this test but without success. Pollen from a number of species of Pyrus growing in the Arnold Arboretum was used, and a variety of media were tried. Usually good germination was secured, but in no case did the tubes reach a length of over 2 mm. We have no satisfactory explanation to offer for this difference.

The present writer has found that pollen from four species of the family Liliaceae will produce tubes on artificial media long enough to effect fertilization if they were growing in the style of the plant. On a 1.5-percent agar medium containing 10 percent cane sugar and a little yeast, excellent growth of the pollen of the grape hyacinth, *Muscari botryoides*, was secured. In the grape hyacinth the styles are about 1.25 mm. in length, and the distance from the stigma to the base of the ovary is about 2.5 mm. but occasionally reaches 3.0 mm. The majority of the tubes on the artificial medium were as long as the style, and a few approached the length of the vertical axis of the gynoecium. The lengths of some of the longer tubes in millimeters were as follows: 1.65, 2.30, 2.04, 1.79, 1.67, 2.02, 1.59, 1.79, 2.20, 1.77.

In Puschkinia, the styles average about 1.5 mm. in length. On a culture medium consisting of 1.5 percent agar, 7 percent cane sugar, and a little sterile yeast, good growth was obtained. The following measurements in millimeters were taken on the longer tubes: 1.41, 1.51, 1.55, 1.57, 1.69, 1.63, 1.55, 1.43.

On the same culture medium, the longer pollen tubes of Chionodoxa, a form in which the style is about 2 mm. long, measured as follows: 2.04, 1.77, 2.18, 1.91, 1.96, 2.00, 2.01 mm.

Excellent growth of pollen tubes of Scilla was obtained on a culture medium consisting of 2 percent agar, 7 percent cane sugar, and a little sterile yeast. Careful measurement showed that the styles of this species range in length from 4.5 to 6.0 mm. The pollen was scattered at random on the culture, and the tubes were grown at room temperature. Fourteen tubes measured as follows: 4.96, 5.14, 5.10, 5.40, 5.34, 4.98, 5.00, 4.92, 5.22, 5.16, 4.75, 6.12, 4.68, 5.40 mm. On this culture there were many shorter tubes, but a very considerable number equal in length to, or even longer than, those whose measurements are given above.

Nuclear Behavior

On the plant, presumably the sole function of the pollen tube is to provide a means of approach of the passive or almost passive sperm nuclei to the embryo sac. The pollen grain germinating upon the stigma gives rise to a pollen tube into which the so-called tube and generative nuclei (or sperm nuclei) pass and make their way to the ovary as the tube extends through the stylar tissue. Stated in these terms, we may say that the results of our studies on artificial media are successful in so far as they promote this nuclear migration. It has already been demonstrated that tubes of sufficient length to reach from stigma to ovule can be secured *in vitro*. It remains to be seen if these have brought their nuclei in position to function were fertilization possible.

Few observations have been made on the behavior of the nuclei in pollen tubes growing on artificial media. The density of the protoplasmic contents of the tubes, with their frequently coarse granular inclusions, makes it usually difficult or impossible in whole mounts to detect the nuclei with certainty. Van Tieghem (1869) was able to get embryos showing the first stages of development on artificial cultures. Ovules in the path of pollen tubes received them through the micropyle and some development ensued. Strasburger (1878), in similar experiments with Torenia, observed fertilization *in vitro*. Elfving (1879) was successful in securing short tubes of several species on sugar media and found that these usually contained two nuclei in their tips. In the pollen tubes of *Plantago media*, Elfving observed and figured three nuclei, one nearest the tip long and narrow, the two others closely behind it, small and oval in shape. The lengths of the tubes in these cases are not given.

In Scilla, a form in which tubes as long as the style of the plant can be secured on agar cultures containing 7 percent cane sugar and a little sterile yeast, the writer found excellent material in which to follow the nuclear behavior in pollen tubes growing *in vitro*. The cytoplasm in the grain and in the tube is very clear. By using Belling's (1921) aceto-carmine, which stain was kindly suggested by Dr. Karl Sax, the large nuclei staining a deep red are sharply differentiated from the more lightly colored cytoplasm.

Observations were made on fresh pollen grains and on tubes at intervals during their growth by removing these from the cultures with a needle and mounting them in the stain. The drawings were made in part with the aid of the camera lucida; the remainder were sketched free-hand. In fresh pollen, as is shown in figure 1, Plate XIX, usually only a single deeply staining nucleus can be detected with certainty. Figure 2 represents the less frequent condition, in which the second and smaller nucleus, although staining faintly, can also be seen. At the time the pollen is shed it is very probable that two nuclei are already differentiated in the grain, although one stains more readily than the other. At the onset of germination the second nucleus is aroused to activity. Usually within 15 minutes after the pollen grain has been placed on the culture the tube is emitted. At this time two nuclei may be clearly observed in the grain (figures 3 and 4). Migration of the nuclei, as shown in figure 5, commonly begins at one hour, or when the tubes have attained a length of about 100 μ. Except in rare cases, the smaller nucleus, presumably the tube nucleus, leaves the grain first and is followed closely by the larger generative nucleus.

In figure 12 are shown pollen tubes pressed out of the stigma of Scilla one hour after pollination. Here, as in the artificially cultured tubes of the same age, both nuclei take the stain and migration has begun. Attempts to follow the pollen tubes in their further growth in the style were unsuccessful, but we may reasonably suppose that in this species the nuclei behave as in the closely related forms which have been studied.

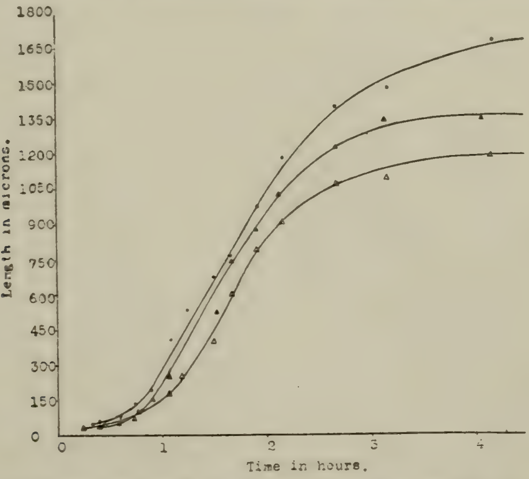
On artificial cultures 1½ hours old, many of the tubes have reached a length of 300 μ. At this stage the tube nucleus is found a short distance from the tip, and the generative nucleus, frequently vermiform in appearance, lies free in the body of the tube as represented in figure 6. In many cases at 5 hours, elongation has ceased and the two nuclei, one closely behind the other (figure 7) are found in the somewhat enlarged tip. Figure 8 represents the tip of a tube about 4 mm. in length taken from a culture after 6 hours. Here the rear nucleus has divided, giving rise to two vermiform sperm nuclei. These do not appear to be accompanied by any specially differentiated cytoplasm. A similar condition is seen in text figure 4, a microphotograph of the nuclei in a Scilla tube 4.24 mm. in length. Here the tube nucleus was 0.37 mm. from the tip of the tube. Each of the sperm nuclei measured about 30 μ in length. No details concerning the division of the generative nucleus to form the two sperm nuclei were obtained. At 7 hours, as shown in figure 9, Plate XIX, in those tubes in which the sperm nuclei have been formed these have become more compactly organized and the tube nucleus is passing into a "resting condition."

In some tubes on 7-hour cultures, various aberrant distributions of the nuclei have been found. A tube 1.37 mm. long is represented in figure 10, one nucleus of which has failed to leave the grain. In another tube (figure 11) 1.43 mm. in length, two nuclei were found near the tip and a third lying near the grain end. In the cases in which the tubes reached an ultimate length equal to only a few diameters of the pollen grain itself, the nuclei usually still remain in the grain. In some instances either the smaller or the larger nucleus is found in the tube, but very seldom both. The relation of nuclear position to pollen-tube length is difficult to analyze. Since, however, some short tubes are found in which the nuclei have taken up their usual position, the writer is inclined to the opinion that the failure of the nuclei to migrate is not *per se* responsible for the limited amount of growth these shorter tubes exhibit. On older cultures pollen grains are occasionally found that have not germinated, in which the nuclei are to all appearances quite normal. Here, as it appears in the shorter tubes, the position of the nuclei is a consequence and not a cause of the failure of the tube to elongate. In a rapidly growing tube, however, where the activity is centered in the tip, we might expect, if the tube nucleus failed to advance to its usual position, that its influence on this growing region would gradually diminish and that growth consequently would be retarded.

The observations as a whole indicate very close agreement between the behavior of the nuclei of pollen tubes growing *in vitro* and those growing *in* *vivo.* In the majority of the tubes growing on the artificial medium, the nuclei migrate into the tubes in the early stages of development and thereafter maintain a relation to the growing tip similar to that found in the styles of such other Liliaceae as have been examined by various workers. On the artificial cultures, moreover, division of the generative nucleus to form the two male gamete nuclei in the longer tubes has been frequently observed. The possession of these facts on nuclear behavior lends further support to the view that culture *in vitro* is a rational method of attack upon the problems of pollen physiology.

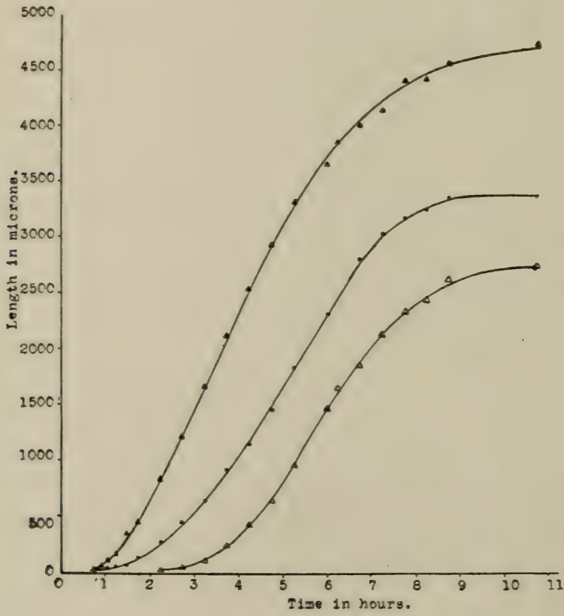
Growth Curves

If some favorable form be chosen, and the pollen grains be plated in such a way that each may be quickly and certainly identified, the length of each of a series of tubes formed can be determined at intervals throughout the growth period. When the values for length are plotted as ordinates over those for time as abscissae, an S-shaped curve of growth is obtained. Pollen of Scilla and Vinca was found quite suitable for this work. Each grows readily on artificial media and pursues a fairly direct course during elongation, facilitating accurate measurement. The cultures were grown in the oven at 27°–28° C., being removed to the stage of the microscope and measured at room temperature. This was subject to some fluctuation, but was generally somewhat lower than the oven temperature. The interval required for measurement being comparatively brief, it is believed that the periodic removal of the cultures from the oven did not alter the character of the growth to an appreciable extent.



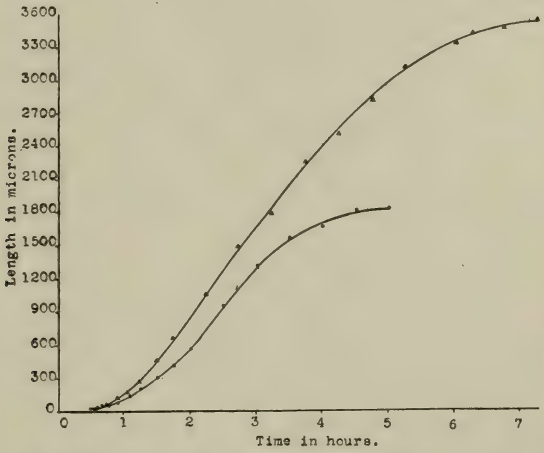
TEXT FIG. 1. Growth curves of pollen tubes of Scilla grown on an artificial medium consisting of 2 percent agar, 10 percent cane sugar, and a little sterile yeast, grown at an oven temperature of 27°–28° C., and measured periodically at a room temperature slightly lower.

In Scilla, the modal length of time required for pollen-germination is about 20 minutes. In a series of 25 pollen grains taken at random, for example, 6, or 24 percent, germinated within 15 minutes after planting, 10 others, or 40 percent of the total, germinated within 20 minutes, 5, or 20 percent, required 25 minutes, and 2, or 8 percent, germinated in 45 minutes: the remaining 2 grains failed to germinate. A potent factor in determining the variability in ultimate length of pollen tubes growing *in vitro* is the bursting of the tubes during elongation. Tubes in any stage of growth may suddenly explode at the tip, and of course cease to grow further. Up to the time of bursting, these tubes can not be distinguished by their behavior from those which survive them. In text figure 1, the growth curves are given of three tubes of Scilla, constituting a fair sample of those whose elongation was not terminated by bursting. They were grown on a culture medium consisting of 2 percent agar, 10 percent cane sugar, and a little sterile yeast.



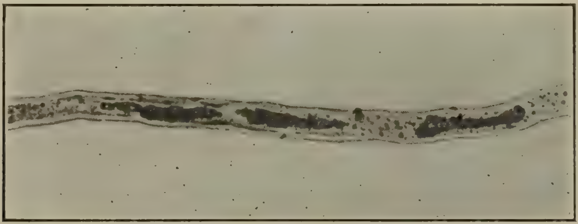
TEXT FIG. 2. Growth curves of pollen tubes of *Vinca minor* grown on an artificial medium consisting of 1.5 percent agar, 10 percent cane sugar, and a little sterile yeast. The tubes were grown at an oven temperature of 27°–28° C. and measured periodically at room temperature, generally slightly lower.

While the ultimate length reached by each of the three tubes represented in text figure 1 differs somewhat, and while there is some variability between them in rate of growth, the type of growth curve given by each is essentially the same. It resembles that of a monomolecular autocatalytic reaction where the amount of substrate is limited. The rate is slow at first, then becomes more rapid, and slows again. There is some tendency toward asymmetry in these curves, the diminution in rate in the later stages being more gradual than the acceleration in the early stages. And, contrary to expectation if the growth follows strictly the course of a monolecular autocatalytic reaction, the maximum rate of elongation does not occur when growth is just half completed. It comes somewhat earlier than this.



Text Fig. 3. Growth curves of pollen tubes of *Vinca minor* cultured *in vitro*, the lower curve representing growth in a yeast-free medium and the upper one growth on a medium containing sterile yeast.

Growth curves were also obtained for the pollen tubes of *Vinca minor* growing at 27°–28° C. on a $1\frac{1}{2}$-percent agar medium, with 10 percent sugar and a little sterile yeast added. In the particular test which yielded the data used in the construction of the curves given in text figure 2, 14 pollen grains selected at random were planted in series. Of these 14 grains, all germinated but one, but 7 of the tubes burst during elongation. The remaining 6 gave rise to tubes which maintained their integrity until their growth cycle had been completed. The three curves given are representa-



Text Fig. 4. Microphotograph of a section near the tip of a Scilla pollen tube grown on a 2-percent agar medium containing 10 percent sugar and a little sterile yeast and mounted directly in aceto-carmine. This tube was 4.24 mm. in length; the tube nucleus (somewhat dumbbell-shaped) was 0.37 mm. from the tip. × 300.

tive of these latter. As in the case of Scilla pollen tubes, the growth curve of *Vinca minor* tubes grown on artificial media simulates that of a monomolecular autocatalytic reaction where the amount of substrate is limited. The significance of the facts presented here in regard to the type of growth prevailing among pollen tubes growing *in vitro* will be considered in conjunction with our observations on the digestion of the reserve materials of the pollen grain during growth, as set forth in the next section.

Further observations reveal the fact that the presence or absence of sterile yeast in the media does not affect the type of growth given by pollen tubes. In text figure 3, growth curves of representative Vinca tubes from yeast-containing and yeast-free media are compared. In the presence of yeast a greater amount of growth is given, but from the character of the curves it is evident that the type of growth is not changed.

Water in its Physical and Chemical Relations to Growth

There is considerable evidence to show that in some forms the rate at which water is delivered to pollen is the most important factor in germination. Jost (1905) found that pollen of Dactylis, Arrhenatherum, Secale, and Glyceria, which could not be induced to form tubes when placed directly on culture media, germinated in a damp room when placed near the media. An examination of the stigmas of these plants confirmed Jost in his idea that the water relation was the most important cultural condition. When viewed under the microscope, no drop of liquid was in evidence on the stigma but only a thin layer at the point of contact with the cover glass. The fact that pollen of Secale and Dactylis germinated on the stigmas of various other species of plants and on the under sides of some leaves, Jost regarded as evidence that germination is dependent upon definite physical conditions. In a later paper, Jost (1907) reports that in Corydalis the only requirement for pollen-germination is a careful control of the water supply. Martin (1913), working with the pollen of *Trifolium pratense*, found that while sugar solutions of various concentrations gave unsatisfactory results, germination could be secured on hog's bladder previously soaked in sugar solutions or in distilled water. Differences in thickness and texture in different parts of the membranes used caused a lack of uniformity in germination. These facts led Martin to the conclusion that the amount of water supplied is the controlling factor in germination. Martin found that the exposed portions of the papillae on the stigmas of Trifolium are covered with a rather heavy cutinized wall. An oily emulsion, such as Strasburger (1884) found in the cuticular cells of the stigmas of Gramineae, was found in these structures on the clover plant. These findings led Martin to the conclusion that "the behavior of the stigma indicates that its function is to regulate the water supply and the nature of the pollen necessitates no other function." Anthony and Harlan (1920), prompted by the results of careful observations in the field at pollination time, were able to germinate the very sensitive pollen of barley by placing it on a slide inside a Van Tieghem cell where moisture was supplied either from a drop of free water or from a piece of mesophyll from the leaf of a garden pea. After the cover glass was put in place the slide was placed out-of-doors on a window ledge. While the humidity of the atmosphere within the cell was coming into equilibrium it was expected that favorable moisture conditions would be met, some moisture would condense on the pollen, and germination would ensue. Under these conditions pollen tubes were obtained in five minutes. Martin (1915), in studying the problem of seed-production in alfalfa, found the pollen of this form very sensitive to moisture. Martin concluded from the results of his experiments that the requirement for germination of the pollen depends upon a certain ratio between the moisture delivered by the stigma and the moisture of the air surrounding the stigma.

Under conditions where the amount of water delivered to the pollen of the above-named forms is not held within narrow limits, bursting usually results. This, as we have discussed in another section, is considered as predominantly an osmotic phenomenon although not entirely independent, perhaps, of the imbibitional processes of the pollen grain. It is quite probable that in the above-mentioned cases the excess water exerted injury by its purely physical effect.

In the culture of pollen *in vitro*, the water in the medium must also be considered in another fundamentally inportant connection, namely, as a component in the hydrolytic and synthetic reactions that attend growth. In following the digestion of the reserve materials by microchemical means in the pollen tubes of *Vinca minor* during growth, the interesting fact was revealed that when the longer tubes have reached their maximum length the reserves are either greatly diminished or have disappeared entirely. In Plate XX, figures 17–23, successive stages are represented in the digestion of the fatty reserve material characteristic of the pollen of Vinca. The tubes were grown on 2-percent agar media containing 10 percent cane sugar and a little sterile yeast. At intervals throughout the period of elongation, tubes were removed from the surface of the agar culture and mounted directly in Sudan III. A sharp differentiation of the fatty particles was thus obtained. A large number of tubes were examined, and, as would be expected, some variability was in evidence among those of a given age both as to length and as to the amount of fatty substance present. In those tubes which fail to grow for more than one or two hours, comparatively little diminution in content of reserve material takes place. Our attention was largely confined to the longer tubes, and the figures given can be considered as fairly representative of the general condition prevailing among these at the various stages.

Germination usually takes place about 45 minutes after the pollen is placed on the medium. Following germination, the coarsely granular contents of the pollen grain pass into the tube as it elongates. Figure 17 represents a tube about 15 minutes old. At this stage the short tube is densely crowded with particles of fatty material of various sizes. While there may be some further accumulation of fat at this time, we are inclined to think that the amounts observed in the tubes are no greater than could be accounted for by the contents of the pollen grain. Thirty minutes later, as is shown in figure 18, many of the smaller fat globules have coalesced, and these larger particles are tending to accumulate toward the tip. From this stage on, the pollen tubes become somewhat more attenuated in appearance, the cytoplasm of the grain and the older portion of the tube gradually becoming clearer through the localization of the fat globules in the region of the tip. In figures 19–23 only the tips are shown, the remaining portions of the tube, as shown in figure 13, being almost or entirely free of granular material. It is readily evident from an examination of figures 17–23, covering a period of about five hours, that there is a diminution progressing with age in the amount of fat in the tubes. Figures 21 and 22, representing tubes from cultures six hours old, show a condition approaching the complete digestion of the stored material contributed by the pollen grain. These reserves are being broken down and utilized in the growth processes. In figure 23 a tube is shown of the same age, in which the fat has entirely disappeared. It is an interesting and significant fact that in such tubes little or no further elongation takes place. Many tubes on these Vinca cultures cease to elongate before their reserves are fully utilized. Factors other than deficient food supply intervene to check growth in such cases. The most frequent cause of the premature cessation of growth is bursting. When a tube becomes broken at the tip, its contents are lost in the medium and its elongation is abruptly halted. The point to be emphasized, however, is that the amount of growth of those which continue to elongate appears to be terminated eventually by the exhaustion of the supply of fatty substance in the tube. Under the conditions of culture *in vitro*, potential length of the tube and the amount of food material initially present in the pollen grain seem to be causally related.

In order to evaluate these findings in regard to the digestion of the reserve food material, it is necessary to bring in review certain facts concerning the kind of pollen-tube growth prevailing on artificial media and in the style of the plant as revealed by the type of growth curve obtained in each case. The studies of East and Park (1918) on the rate of pollen-tube growth in the style of Nicotiana revealed the fact that after a compatible cross the curve obtained by plotting lengths of tube as ordinates and time as abscissae simulated that of an autocatalytic reaction. In the writer's opinion it would be justifiable to add "where the substrate was present in excess," since the rate of growth in the later stages shows no sign of diminution; the curves so far as plotted do not "turn over." In some successful grafting experiments, Jost (1907) demonstrated that pollen tubes would grow through two styles placed end to end; that is, pollen tubes can grow much longer than is necessary to effect fertilization. There is no reason to suppose that the position of the pollen tube in the style has any influence, *per se*, on the rate of growth. In so far as the physical impediments interposed by grafting would permit, it is very probable that in Jost's experiments the tubes traversed the styles in a single cycle of growth. If the rate were diminished as the tubes neared the end of the first style through a lack of food material, it would be unlikely that they could have grown through and even projected beyond the second one. The evidence afforded by Jost's experiments and those of East and Park support the view that in the style of the plant there is no diminution in rate of growth in the later stages such as would be expected were there a deficiency of substrate.

As shown in a previous section, the growth curves of pollen tubes grown on artificial media resemble that of an autocatalytic reaction, which, according to the commonly accepted interpretation, is slower in the initial stages because of a deficiency of catalyst and in the final stages because of a deficiency of substrate. One further fact is to be noted here, namely, that as shown in *Vinca minor* the reserve material of the tube is practically exhausted when the tube has reached its maximum length. Loeb (1906) contended that the synthesis of nuclear material, a conspicuous feature of growth by cell division, is an autocatalytic process and that the nucleus itself or one of its constituents acts as the catalyser. Robertson (1908 *a*, *b*) expanded this idea and sought to show that data on the growth of man, of *Cucurbita pepo*, and of fresh and dried oats are graduated in a satisfactory manner by the curve for an autocatalytic reaction. It is concluded that the agreement found is good, and Robertson sets forth the view that, in all probability, cell growth, or the synthesis of cytoplasm, is an autocatalytic reaction.

It has not seemed pertinent with the somewhat limited amount of data available at this time to determine if satisfactory graduations are obtained when the theoretical curve for an autocatalytic reaction is fitted to the observations on pollen-tube growth *in vitro*. The evidence presented, however, is suggestive that this type of curve may adequately describe the quantitative relations of the phenomenon. Pearl (1909) has pointed out the fallacy of supposing, in the absence of confirmatory evidence of another sort, that phenomena involving quantitative changes described by the same type of curve have a common cause; qualitative proof must be furnished to establish finally the qualitative identity of phenomena. The growth curves presented above, representing graphically the quantitative relations obtaining in pollen-tube growth on artificial media, are supplemented by observational evidence on the digestion of the reserve food material during elongation of the vegetative cell. There is little doubt that growth is causally related to the digestion of the food substance within the tube; that the form of the curve describing growth is wholly dependent upon the course of the reaction involved in the digestion of the reserve food substance, is less certain. That pollen-tube growth is an autocatalytic process dependent directly on the hydrolytic and synthetic reactions involved in the production of food material in the tube, however, is a simple and attractive explanation of the phenomenon. Let us see how it fits the known facts regarding pollen-tube growth on artificial media and in the style of the plant.

In monomolecular autocatalytic reactions, it is assumed that one large molecule, the substrate, is undergoing a process of being divided up into smaller ones under the action of a catalyst. In the case of pollen-tube growth the substrate is qualitatively the same throughout the whole growth cycle, and its nature is revealed by the character of the reserve food materials in the pollen grain. The catalyst is an appropriate enzym. In the form used we are dealing with a fat$\rightleftharpoons$glycerol + fatty-acid system, and the reaction would be represented thus:

$$\text{fat} + \text{water} \overset{\text{enzym}}{\rightleftharpoons} \text{glycerol} + \text{acid}.$$

This is, of course, a reversible reaction, the enzym accelerating it in both directions. Now, if we consider the equilibrium positions of this system under different conditions, we find a very plausible explanation of the facts at hand regarding pollen-tube growth. The knowledge that reactions in the living cell are reversible presupposes a mechanism for controlling the amount of water in the system. If this were not so, all reactions could go in but one direction. In pollen-tube growth *in vivo*, the reserve material, fat or starch as the case may be, hydrolyzed in the process is replaced directly or indirectly from sugar derived from the stylar tissue. Growth is a continuous process of hydrolysis and synthesis of fat in the tube, the rates being determined by the masses of the reacting substances. If the mass of the water component of the system were low, the reverse reaction could proceed as rapidly as hydrolysis. Under these conditions the effect due to a deficiency of substrate would not be observed, and we should get a growth curve such as East and Park (1918) found for pollen tubes in the styles of Nicotiana. On the other hand, on the artificial cultures the pollen tubes are growing in a watery medium. Owing to the excess of water present, the equilibrium position in the system:

$$\text{fat} + \text{water} \overset{\text{enzym}}{\rightleftharpoons} \text{glycerol} + \text{acid},$$

is very near complete hydrolysis. The amount of synthesis is too small to be perceptible; that is, the reaction goes almost entirely to the right and terminates, as does growth, when the substrate is exhausted, giving an S-shaped curve.

Thus far we have not considered the influence of the sugar present in the artificial medium. It has already been noted that there is a more or less general tendency toward asymmetry in the curves and that the maximum rate of growth especially in Scilla does not occur when growth is just half completed. It seems to come somewhat earlier in the cycle, and the curve in its later stages shows a slowly decreasing growth rate. The simplest interpretation of this asymmetry is that the sugar supplied in the artificial medium supplements the food materials formed by hydrolysis of the reserves and thus prolongs growth especially in the later stages. If the sugar is actually a component of the reaction system, such an effect would result from its mass action. The position of equilibrium, however, would not be materially altered, because of the great preponderance of water in the system.

Green (1894), working with lily pollen, has demonstrated a rapid increase in the quantity of enzym present during germination and the early stages of growth. It may well be that this is the cause of the increasing rate of growth at this stage. There are reasons other than exhaustion of the reserve food material in the pollen tube, however, that might account for the decline in growth in the later stages. Among these we may mention two: (1) exosmosis of some essential constituent of the cell; and (2) accumulation of products of metabolism having a toxic effect.

The hypothesis that pollen-tube growth on artificial media is an autocatalytic reaction in which the substrate is limited seems to interpret in a plausible manner the important difference (if we may generalize on the one hand from the single but carefully analyzed case of East and Park (1918) on pollen-tube growth in the style of Nicotiana) which exists between pollen-tube growth *in vitro* and *in vivo*. If this difference is dependent, as our hypothesis holds, upon the difference in the water relations in the two cases, we should be able by varying this factor in the artificial cultures to change the shape of the growth curve. Only on artificial media containing a minimum of water could we expect to get a growth curve similar to that found by East and Park. Definite conclusion as to the adequacy of our hypothesis to interpret pollen-tube growth must await the results of such experiments.

EXPLANATION OF PLATES

PLATE XIX

Distribution of the nuclei in the pollen tubes of Scilla grown on a medium consisting of 2 percent agar, 7 percent cane sugar, and a little sterile yeast. Figures drawn from whole tubes mounted in aceto-carmine. The drawings in part were made with the aid of the camera lucida; the remainder were sketched free-hand.

FIG. 1. Pollen grain in the resting condition, showing a single nucleus. × 400.

FIG. 2. Pollen grain in the resting condition, showing two nuclei. × 400.

FIGS. 3, 4. Germination at 15 minutes. × 400.

FIG. 5. Migration of nuclei from the pollen grain at 1 hour. × 400.

FIG. 6. Position of the nuclei at $1\frac{1}{2}$ hours. × 300.

FIG. 7. Tip of fully grown tube at 5 hours, showing the two nuclei in the tip. × 300.

FIG. 8. Tip of tube 6 hours old, showing the tube nucleus and two sperm nuclei. × 300.

FIG. 9. Tube nucleus and two sperm nuclei at 7 hours in the tip of a long tube. × 300.

FIG. 10. Tube 1.37 mm. long at 6 hours, showing aberrant distribution of the nuclei. × 300.

FIG. 11. Tube 1.43 mm. long at 6 hours, showing unusual distribution of nuclei. × 150.

FIG. 12. Pollen tubes removed from the style at 1 hour.

PLATE XX

Figures 13–16 were drawn from pollen tubes of *Vinca minor* growing on an artificial medium. Figures 17–23, showing the digestion of the reserve fatty material, were drawn from pollen tubes of *Vinca minor* grown in a medium composed of 2 percent agar, 10 percent cane sugar, and a little sterile yeast. The tubes were mounted in Sudan III and sketched free-hand. Magnification, 200 diameters.

FIGS. 13–16. Four stages in the development of callose plugs.

FIG. 17. Pollen tube from a culture 1 hour old, or about 15 minutes after germination.

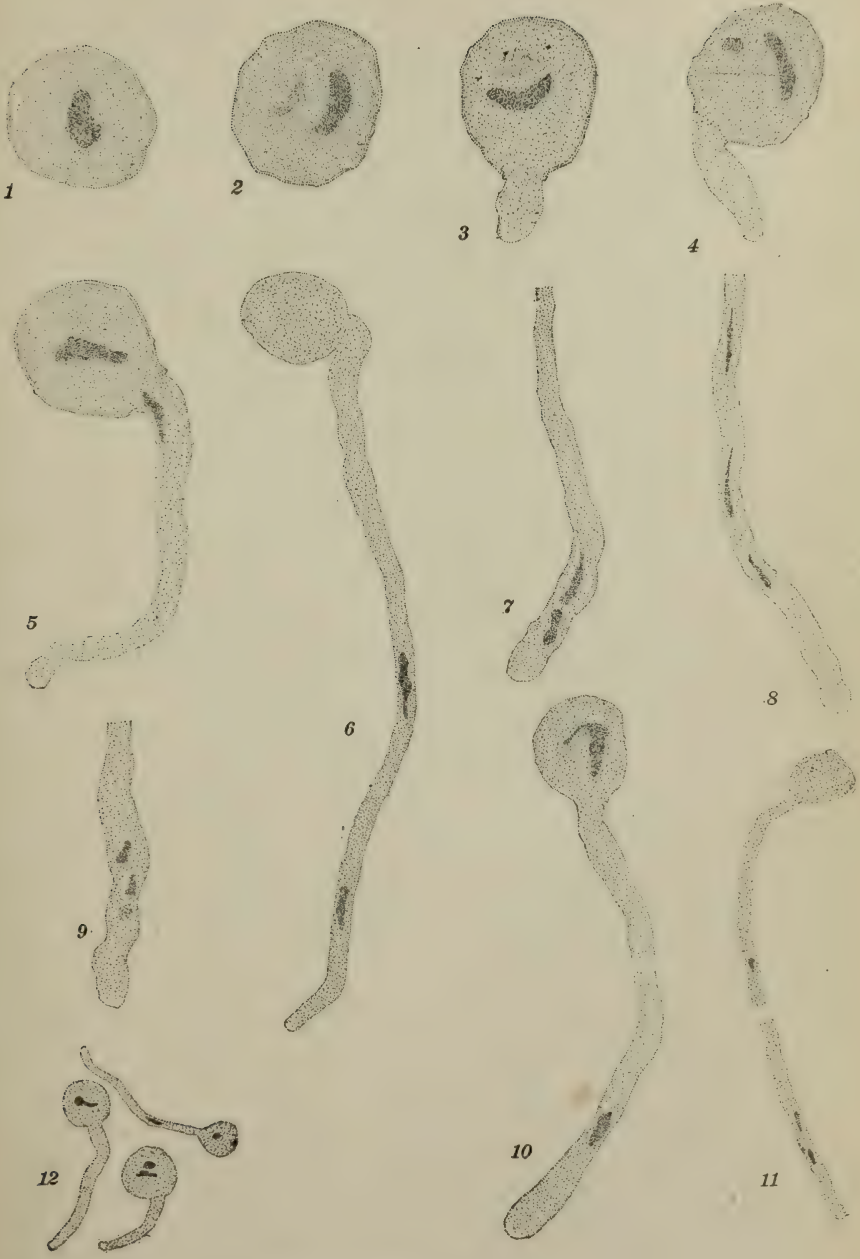
FIG. 18. Pollen tube from a culture 1½ hours old.

FIGS. 19, 20. Tips of pollen tubes at 3 hours.

FIGS. 21, 22. Tips of long pollen tubes from culture 6 hours old, showing advanced stages in the digestion of the reserve food material.

FIG. 23. Tip of long pollen tube at 6 hours, in which the reserve material has been entirely digested. A single nucleus is shown in the tip.

FIG. 24. Pollen tube of *Cucurbita pepo* grown *in vitro*, showing the branching habit. × 40.



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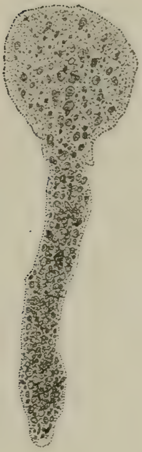
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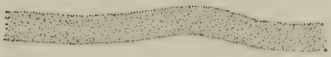
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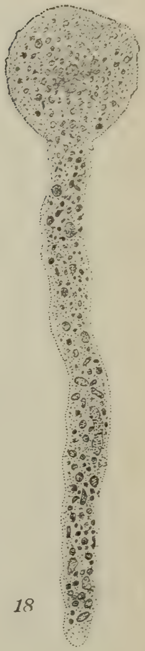
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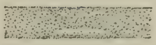
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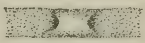
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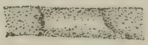
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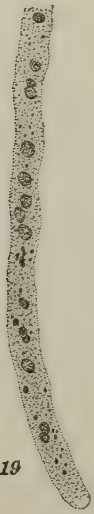
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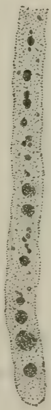
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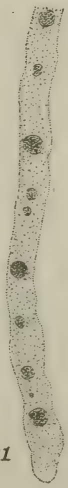
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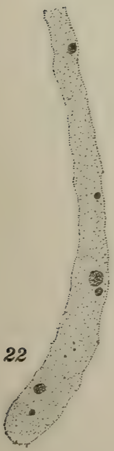
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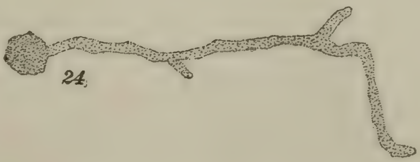
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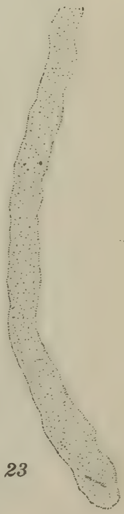
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BRINK: PHYSIOLOGY OF POLLEN

Reprinted from the American Journal of Botany, XI: 417–436, July, 1924.

THE PHYSIOLOGY OF POLLEN

IV. CHEMOTROPISM; EFFECTS ON GROWTH OF GROUPING GRAINS; FORMATION AND FUNCTION OF CALLOSE PLUGS; SUMMARY AND CONCLUSIONS[1]

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(Received for publication July 5, 1923)

Chemotropism

In seeking an explanation of the direction of pollen-tube growth in the pistil of the plant, three different possibilities suggest themselves. The tube may be passively guided from the receptive surface to the micropyle of the ovule by certain anatomical features of the tissue traversed; it may be oriented in its course by the diffusion from particular centers of substances having a chemotropic effect; or, when growing over free surfaces, the pollen tube may arrive at the micropyle purely as a matter of chance.

A rather extensive literature pertaining to this subject has grown up, concerning especially the tropistic reactions of pollen tubes. This we shall review briefly. Van Tieghem (1869) regarded hydrotropism as the cause of the pollen tube's entering the stylar tissue. Working with a large number of plants, but particularly with Torenia, Strasburger (1878) found no evidence of chemotropism for ovules. Pollen tubes growing in drop cultures showed no tendency in the presence of ovules to grow into the micropyles more frequently than in any other direction, and usually, upon coming in contact with ovules, grew over them and separated from them on the other side. In search of an explanation of the incompatibility of matings in heterostyled plants, Correns (1889) found no difference in chemotropic reaction between the pollen from the two forms of *Primula acaulis*, nor indeed was any chemotropism demonstrated in this species.

Molisch (1893) reported that the pollen tubes of *Narcissus tazetta* are negatively aërotropic. When cultured in sugar solutions under a cover glass, only the grains in the peripheral region germinated and these sent their tubes inward toward the region of lower oxygen tension. Precautions were taken to prevent evaporation at the edge of the cover glass. Molisch states, however, that this phenomenon was not general. In the same species it was found that the stigma, parts of the petiole and flower, the micropyle and funiculus of the ovule, and stigmas from other species exerted a chemotropic effect on pollen tubes growing in their vicinity. Molisch concluded that in Narcissus the female sex apparatus through definite secretions calls forth a chemotropic response in the pollen tube which leads it to the egg.

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[1] Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

Miyoshi (1894) regarded chemotropism for the stigma as a general phenomenon. He reports having seen drops of liquid at the micropyle which he believed were secretions that attracted the tubes. Some sugar, possibly sucrose, was thought to be the active agent. It was demonstrated that pollen tubes are indifferent to light and that geotropism plays no rôle in determining the direction of their growth. On cutting off the style of *Digitalis purpurea* and pollinating the cut end, it was observed that some tubes grew into the air, whereas others grew in the reverse direction and their tips appeared ultimately at the tip end. If the stigma was cut off or damaged, the tubes grew and projected still more. These results eliminated chemotropic phenomena as possible causes of orientation in the style and led Miyoshi to the conclusion that pollen tubes follow the line of least resistance to the ovary. In the ovarian cavity, however, a chemotropic stimulation accounts for their entry into the micropyle.

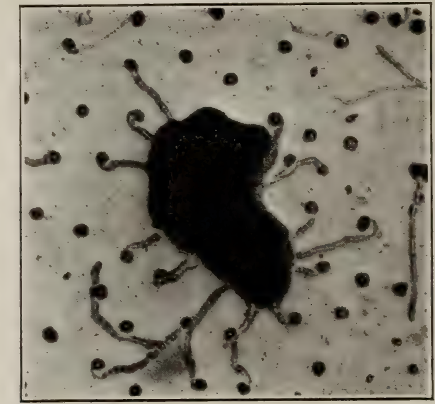
Lidforss (1899 *a*) confirmed Molisch's observation that Narcissus tubes cultured on 5–10 percent sugar-gelatin media grow toward the stigma. He did not believe, however, that the active substance was a carbohydrate; nor could he demonstrate any chemotropic effect toward organic acids, such as formic, acetic, lactic, succinic, malic, tartaric, or citric acid. A bit of onion root exercised a strong chemotropic effect, which fact indicated to the author that the active substance was widely distributed. Diastase, even after boiling or treatment with sulphuric acid, exerted a pronounced chemotropic effect, suggesting to Lidforss that some protein substance was concerned. Dialyzed albumin attracted Fritillaria tubes. Protein derivatives, such as tyrosin, were without effect. Lidforss believed that the chemotropic effect of these protein substances probably plays an important rôle in the passage of the pollen tube through the style. Kirkwood (1906) considered the embryo sac the source of a stimulant of some sort, probably a sugar, by which the pollen tube is directed unerringly toward it.

Tokugawa (1914), following Lidforss, divided pollen into two groups, namely, "saccharochemotropische" and "proteochemotropische." It was shown that Narcissus pollen tubes were attracted to the stigmas of Prunus and *vice versa*, but that often this reciprocal relation did not hold. Working with Nicotiana, East and Park (1918) found no certain evidence of positive chemotropism for parts of the gynoecium. Knowlton (1922) observed that the pollen tubes of Antirrhinum growing on artificial media grew toward and even penetrated stigmatic tissue placed on the culture. Pollen tubes of apple were similarly attracted to the stigma of the apple, but in this form the phenomenon was less marked.

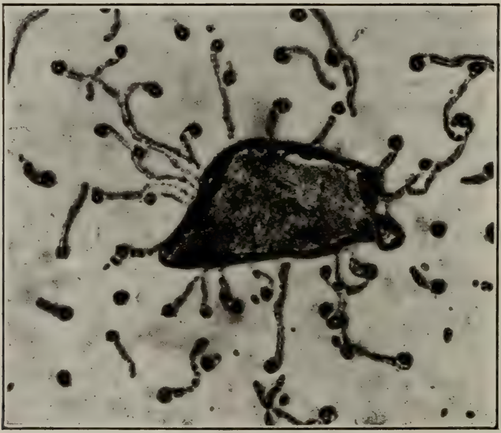
Anatomical studies led Capus (1878) to the view that no particular arrangement exists which facilitates the entrance of the pollen tube into the micropyle and that some undetermined physiological phenomenon causes the tube to be attracted to its objective. Dalmer (1880), on the other hand, quotes Behrens (1875) as having found that the long walls of the stylar cells are easily separated from each other while the cross walls possess a marked tenacity. Where a stylar canal was present, Behrens found that its outer layer had secretory functions. Dalmer expressed the view that the distribution of the conducting tissue in the ovary depends wholly on the position of the micropyle. In Polygonum and Daphne, where the micropyle lies directly at the base of the style, no conducting tissue is developed. In forms where the micropyle is not so conveniently situated, it was found that the surfaces leading to it are covered with papillose cells. Dalmer dissents from Capus' view that some physiological phenomenon must be invoked to explain the passage of the tube to the micropyle, and holds that in the carefully and fully studied cases the conducting tissue has been found to extend to the micropyle. The pollen tube, in his opinion, is forced by outside conditions to enter the micropyle. Guéguen (1901), after an extensive study of the anatomy of the style, concluded that in monocotyledons the distribution of the conducting tissue is entirely dependent upon variations in number and position of the ovules. It is in the Liliaceae that the greatest simplicity of conducting apparatus is found. Here stigma passes to stylar canal and stylar canal to the conducting tissue of the ovary walls and placenta very gradually. Among the dicotyledons the conducting tissues show greater variability and complexity, but of all the regions traversed by the pollen tube after its emission on the stigma, the ovary presents the least variation in the structure of the conducting apparatus. In the great majority of cases, the rôle of conduction devolves upon the epidermis of the internal face of the carpellary leaf or on the epidermis of the placenta. Guéguen investigated the subject from the phylogenetic point of view and gave comparatively little attention to the physiological considerations involved.

During the course of the author's investigation, the various forms of pollen used have been tested for their chemotropic reactions to various plant tissues, principally gynoecium parts. In only two species, Antirrhinum and the paper-white Narcissus, was the phenomenon of chemotropism demonstrated. Text figures 1, 2, and 3 show pollen tubes of Narcissus growing toward placenta, epidermis, and ovule respectively of the same form on a culture medium consisting of 2-percent agar, 15-percent cane sugar, and a little sterile yeast. Pollen tubes of Antirrhinum were also found to be attracted toward their own stigmas. In Hippeastrum, Scilla, Nicotiana, Cucumis, Primula, Lythrum, and Vinca, pollen tubes growing on artificial media showed no tropistic reaction to gynoecium parts.

In conjunction with these tests for chemotropism to bits of plant tissue, experiments were made to determine if the pollen tubes of the various species were attracted by cane sugar or by sterile yeast. Agar plates were prepared carrying varying proportions of sugar and yeast, and small sections of the semi-solid medium were cut from these in such a way that two blocks each derived from a different medium could be fitted together so that their upper surfaces were practically continuous. Pollen was distributed with a needle on either side of the junction of the two blocks, and the direction the tubes took during growth on the various combinations of blocks was noted. As was expected, germination of all the species tested



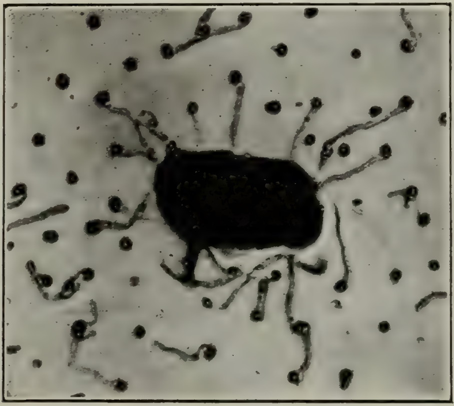
Text Fig. 1. Pollen tubes of paper-white Narcissus grown on an artificial medium, showing chemotropism for placental tissue.



Text Fig. 2. Paper-white Narcissus pollen tubes grown on an artificial medium, showing chemotropism for epidermal tissue from the internal wall of the ovary.

was not secured on each medium. In a considerable number of cases, however, good growth on both blocks of the pair was secured. On pairs of blocks containing the respective concentrations of sugar, 0–3, 3–10, 0–10, 0–20, 3–20, and 10–20, the pollen tubes of Cucumis, Primula, Hippeastrum, and Nicotiana grew entirely at random. Differences in concentration of sugar in the adjacent areas occasioned no orientation of the tubes. Similarly, on pairs in which the differential was sterile yeast, the tubes appeared to grow entirely at random.

Using Pfeffer's method of fine capillary tubes filled with the solution being tested and closed at one end with paraffin to retard diffusion, marked chemotropism of Narcissus pollen tubes for sodium malate has been found. Further experiments are in progress with salts of malic acid.



Text Fig. 3. Pollen tubes of paper-white Narcissus, showing chemotropism for an ovule on an artificial medium.

It is interesting to note, in connection with text figures 1, 2, and 3, that the pollen tubes of Narcissus are attracted not only by the ovules but also by the placenta and by pieces of the epidermis from the internal wall of the carpel. A quantitative method of determining the relative chemotropic values of these different tissues has not yet been devised, but it is quite obvious that, unless the ovule exerts a greater attraction to pollen tubes in the ovarian cavity than other parts, chemotropism can play no rôle in directing the pollen tube to the micropyle.

The Effects on Growth of Grouping Grains and the Problem of Self-sterility

Early in our work it was observed that tubes arising from groups of grains on artificial media were longer than those produced by single isolated grains. Attention was first directed to the condition in cultures of Nicotiana, where it regularly appeared that the longest tubes came from large masses of pollen grains. Casual observations on other forms indicated a similar situation and it was decided to put the matter to careful experimental test. Cucumis pollen was chosen as giving high germination and as being of sufficient size to be quickly and conveniently plated as required. The method of testing was simple. The groups, consisting of four grains in a cluster, were arranged on one side of the culture and spaced to avoid overlapping of the tubes of adjacent groups during growth. On the same culture, parallel to the series of groups but remote from them, lines of single grains were similarly arranged. The tubes were measured when elongation had ceased.

A greater amount of manipulation with the needle was required to plate the single grains than the quartets, and it was at first thought that perhaps a difference in the amount of injury effected was responsible for the difference in growth. It was found, however, that such was not the case. Groups composed of grains previously singled out still gave greater growth.

TABLE 1. *Summary of the Results of 5 Tests on the Relative Amounts of Growth from Pollen Grains Spaced Singly and from Groups of 4 on Agar Cultures Containing Cane Sugar (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of tubes Measured	Average Length	No. of tubes Measured	Average Length	
1	91	343	95	466	123
2	50	338	104	563	225
3	126	323	91	476	153
4	120	502	119	659	157
5	69	261	70	410	149
Average	456	366	479	529	163

In table 1 the results of five tests on the relative amounts of growth from single grains and from groups of four on agar cultures containing only cane sugar are set forth. In each test the average growth from the quartets of pollen grains exceeded that from the singles; the differences range from 123 μ to 325 μ. The average difference, considering all the tubes measured in each series, is 163 μ, or an increase in growth due to grouping of 44.5 percent.

The results of six similar tests on the same medium with sterile yeast added are given in table 2. Here the results are somewhat more variable, but the growth from the quartets is on the average clearly in excess. In test 4, the average length of the tubes in the series of singles exceeded that in the quartets by 236 μ, a result we can not account for. The series of tests on yeast media involved the measurement of 1063 tubes, approximately equally divided between singles and quartets. The tubes from the latter averaged 255 μ longer than those from the single grains, a 34.6-percent increase in growth attributable to grouping.

TABLE 2. *Summary of Results of 6 Tests on the Relative Amounts of Growth from Pollen Grains Spaced Singly and from Groups of 4 on Agar Cultures Containing Cane Sugar and a Little Sterile Yeast (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	136	522	95	563	41
2	65	466	88	947	481
3	59	707	81	1331	624
4	91	1270	111	1034	−236
5	71	932	121	1254	322
6	78	758	67	1055	297
Average	500	768	563	1034	266

Groups of four grains each were used largely as a matter of convenience; with larger groups, the accurate determination of the length of each tube was more difficult. It was found, however, that with some forms groups of four did not give an appreciably greater average amount of growth than grains spaced singly. Very probably the small size of the pollen grains in these species and the rather rangy growth of their narrow tubes accounts for this, since in such cases if the size of the group was increased the effect due to grouping was unmistakably shown. None of the forms tried failed to respond under such conditions, although, with such species as Lythrum, groups of less than ten grains failed to give a measurable effect.

In order to determine if the substance or substances contributing to increased growth in groups of tubes were present as such in the pollen grains, quantities of fresh pollen were removed from anthers and after being crushed in a mortar were scattered on the agar plates either somewhat uniformly over a narrow strip or in small heaps fairly uniform in size. Single whole grains were placed at regular intervals among these, and the

TABLE 3. *Summary of the Results of Experiments to Determine if Pollen Tubes Give Greater Growth in the Presence of Crushed Pollen Grains (Lengths in Microns)*

Test	Without Crushed Grains		With Crushed Grains		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	39	1387	39	1434	+ 47
2	49	824	33	502	−322
3	20	609	22	809	+200
4	87	404	68	734	+330
Average	195	727	162	865	+138

amount of growth they gave was compared with that of single pollen tubes growing on portions of the same plate free from the crushed material. The results of four tests with Cucumis pollen are given in table 3.

In three of the four tests reported in table 3, the tubes growing among crushed pollen grains gave a greater average growth than those on the plain medium. In the other test the latter tubes were longer. Little significance can be attached to these results, however, for it was found upon careful examination that the method used in preparing the crushed pollen was faulty; in the process of crushing many grains were not broken, as revealed by their ability to swell when placed on the culture and to germinate in some cases. The experiment was carefully repeated with large numbers of grains, great care being taken to obtain thoroughly crushed material for the tests. After this was scattered, any grains not broken in the attrition process were removed with a needle before the grains whose tubes were to be measured were placed on culture. The results of the tests when these precautions were observed are given in table 4.

TABLE 4. *Summary of Results of Experiments to Determine if Pollen Tubes Give Greater Growth in the Presence of Crushed Pollen when Special Precautions are Taken to Secure Thorough Breaking up of the Grains (Lengths in Microns)*

Test	Without Cr[illegible]d Grains		With Crushed Grains		Difference
	No. of Tubes	[illegible]e Length	No. of Tubes	Average Length	
1	120	[illegible]	156	789	− 2
2	110	[illegible]	108	481	+98
Average	230	[illegible]	264	663	−27

It is evident that, when the concentration of pollen killed by crushing is increased on the culture, greater growth does not result. The substance or substances promoting increased growth, whatever their nature may be, are apparently products of the metabolism of the pollen tube; they are readily diffusible and are utilized more completely when the tubes are massed.

The point having been established that the grouping of grains conduces to a greater amount of growth, the question was asked whether this was due to an acceleration of the rate from the start or to a prolongation of the growth period. A knowledge of their effect upon rate of growth might afford a clue to the nature of the substances at work. Accordingly, an experiment was arranged to determine the respective rates of growth of pollen tubes growing singly and when grouped with others. Each group consisted of nine grains, among which the single one whose behavior was to be followed was introduced after the others had attained a length of about 200 μ. Since the growth-promoting substances appeared to be products of metabolism, it was thought that this procedure would insure their presence in an effective concentration from the start. The series of isolated grains was arranged on the same agar plate some distance from the groups. In table 5 the average times required for the tubes in the two series to reach lengths of 410 μ and 710 μ respectively are given.

TABLE 5. *Rates of Growth of Cucumis Pollen Tubes Grown Singly and in Groups*

	No. of Tubes	Time in Minutes Required to Reach Length of 410 μ	No. of Tubes	Time in Minutes Required to Reach Length of 710 μ
Singles . . .	10	45	6	76
Groups . . .	10	46	6	74

As shown in table 5, the rates of growth of isolated tubes and of tubes from groups of Cucumis pollen grains are not significantly different up to the stage when each series reaches a length of 710 μ. The experiment was repeated with pollen from the same form, with essentially similar results. It is beyond this stage that the difference in rate of growth becomes evident; the growth of the isolated tubes soon slows down and finally ceases, while the tubes from the groups continue to elongate steadily until eventually their growth also subsides. It should be pointed out in connection with this test that Cucumis pollen tubes grow very rapidly; that they were grown on a watery medium and that the excess of water, influencing the growth rate to a considerable degree as it must, probably obscures any accelerating effect of the substances in question. With a form in which the pollen tubes grew more slowly than those of Cucumis on artificial media, it was thought that any acceleration might be more easily detected. Pollen of *Vinca minor* fulfilled this condition and was used in a single experiment arranged as before. The results are given in table 6.

TABLE 6. *Rates of Growth of Vinca Pollen Tubes Grown Singly and in Groups*

	No. of Tubes	Average Time in Minutes Required to Reach Length of 200 μ	No. of Tubes	Average Time in Minutes Required to Reach Length of 200 μ
Singles . . .	8	54	3	140
Groups . . .	12	40	8	68

Germination was rather poor in this experiment, and growth was irregular. Made at the end of the season, want of further pollen unfortunately precluded its repetition. The differences in rate of growth incident upon grouping appear to be very striking, but, as is shown, the values are based on small numbers of tubes. If further experiments bear out these results, we might conclude with considerable justification that the growth-promoting substances whose activities are revealed by grouping are catalytic in nature.

As shown by the studies of Jost (1907), Martin (1913), and East and Park (1918), self-sterility results from the failure of the pollen tubes after an incompatible pollination to grow fast enough in the style to reach the ovary and effect fertilization during the life of the flower. The cause of sterility is to be sought among the factors affecting the rate of growth. Various theories have been advanced to account for this condition. Jost (1907), in seeking an interpretation of the phenomenon, has postulated "Individuellestoffe," specific substances in self-sterile plants which inhibit pollen-tube growth. The sterility relations prevailing among the heterostyled forms, Jost believes, are best interpreted by assuming quantitative differences in the "Individuellestoffe." Correns (1912) advanced a somewhat similar hypothesis. East and Park (1918) have interpreted the facts in a different way. These authors state that:

> Pollen tubes in a selfed pistil are not inhibited in their growth by substances secreted in that pistil, but rather that a substance or substances are secreted in the pistil after a compatible cross which accelerate growth, and that the direct cause of this secretion is a catalyser which the pollen-tube nucleus is able to produce because the zygotic constitution of the plant producing it is different in certain particular hereditary factors from that of the plant on which it is placed.

On this hypothesis, there is some local reaction between the pollen tube and the adjoining cells whereby the nutrients necessary for tube growth are made available. It is pointed out that the effect must be purely local, since the presence of compatible pollen tubes does not accelerate the growth of incompatible ones.

Put in its simplest form, an adequate interpretation of the facts regarding pollen-tube behavior in self-sterile forms must explain why, in the self-sterile but cross-fertile plants *A* and *B*, the two individual pollens react differently to the stylar environment of each plant. If the growth-promoting substances diffusing from pollen tubes, as demonstrated in artificial cultures, play the essential rôle in determining fertility or sterility (on the plant), they should exhibit the specificity that these relations demand; tubes from the pollen of *A* and *B* respectively should produce substances that are qualitatively different. Are we dealing here with a single substance produced in common by all pollen tubes, or with a class of substances alike only within compatible groups? It is upon the answer to this question that we must rest our judgment regarding their significance in self-sterility.

In investigating this matter, the most suitable material perhaps would be pollen easily cultured *in vitro* and from a self-sterile species in which the self-sterility relations had been determined. Pollen satisfying both these conditions, however, was not available, and the experiments were made with pollen fulfilling the former requirement in a satisfactory way and taken from two widely different species, namely, *Cucumis sativus*, a cucurbit, and *Vinca minor*, belonging to the Apocynaceae. If the growth-promoting substances produced by pollen tubes are specific in their nature, the growth of neither should be stimulated in the presence of the other.

Preliminary tests were made in order to determine approximately the size of group required in the case of Vinca pollen to give a decided increase in growth. The results as summarized in table 7 show that clusters of four grains give a markedly greater growth than isolated grains.

TABLE 7. *Growth of Vinca minor Pollen Tubes Grown Singly and in Quartets (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	79	398	86	654	255
2	87	359	93	767	408
Average	166	378	179	712	355

The difference, amounting to 88.8 percent, is abnormally high perhaps, but the point is clearly established that the large, vigorous tubes of Vinca freely produce some substance or substances which stimulate their own growth at least. Will these substances stimulate the growth of the pollen tubes of Cucumis?

TABLE 8. *Growth of Cucumis Pollen Tubes when Grown Singly, in Quartets, and in Groups of Vinca Pollen Tubes on Sugar-agar-yeast Media (Lengths in Microns)*

Test	Singles		Quartets		Singles in Groups of Vinca Tubes	
	No. of Tubes	Average Length	No. of Tubes	Average Length	No. of Tubes	Average Length
1	124	459			51	522
2	112	453	123	437	61	685
3	81	1142	103	1134	26	2032
Average	317	631	226	755	138	879

To answer this question, seven carefully repeated tests were made, three on a 2-percent agar medium containing ten percent cane sugar and a little sterile yeast, and four tests on a medium lacking the latter constituent. In each test except one, three series of Cucumis pollen grains were arranged on a single agar plate, the first series consisting of isolated grains, the second of clusters of four; in the third series, groups of four Vinca pollen grains were arranged in succession, and after these had germinated a single Cucumis pollen grain was inserted in each group. When elongation had ceased, the average length of the Cucumis pollen tubes was determined for each series. The results of the tests on the yeast and yeast-free media are given in tables 8 and 9 respectively.

Table 9. *Growth of Cucumis Pollen Tubes when Grown Singly, in Quartets, and in Groups of Vinca Pollen Tubes on Sugar-agar Media (Lengths in Microns)*

Test	Singles		Quartets		Singles in Groups of Vinca Tubes	
	No. of Tubes	Average Length	No. of Tubes	Average Length	No. of Tubes	Average Length
1	104	384	133	453	58	394
2	79	379	97	628	49	1040
3	150	241	108	328	53	335
4	112	443	108	796	69	826
Average	445	350	446	544	229	649

It will be noted in table 8 that in tests 2 and 3 the average growth of Cucumis pollen tubes in quartets is slightly less than in the series from isolated grains. The difference is very slight, however, and, when the first test which did not involve a series of grouped Cucumis grains is considered, the average of the tests shows a balance in favor of the quartets of 19.6 percent, a value which more nearly approximates the difference we should expect in view of the results of several other similar tests previously made. In all the other tests the Cucumis groups gave a greater growth than the isolated grains; moreover, in all the tests but one the single Cucumis grains growing in groups of Vinca tubes gave greater average growth than either of the other two series. The differences in the amount of growth of Cucumis pollen under the three different conditions is clearly illustrated in table 10, where the amount of growth of the isolated grains on the two kinds of media is taken as 100 in each case, that of the other two series being given in proportion.

Table 10. *Comparative Amounts of Growth of Cucumis Pollen Tubes when the Grains are Isolated, in Quartets, and Inserted Singly in Groups of Vinca Pollen, the Amount of the First being Taken as 100*

Medium	Singles	Quartets	Vinca Groups
Sugar-agar-yeast	100	119.6	139.3
Sugar-agar	100	155.4	185.4

The increase in growth of Cucumis pollen tubes in the vicinity of Vinca tubes is very striking. Differences of 39.3 percent and 85.4 percent on yeast and yeast-free media respectively are undoubtedly significant. Contrary to expectation if the growth-promoting substances are specific in their action, it has been found that those produced by one species stimulate the growth of tubes of a distantly related one. The excess of growth of Cucumis tubes in the Vinca groups over the quartets of Cucumis pollen is possibly due to the comparatively greater growth of the Vinca tubes, with a consequent increase in the production of growth-promoting substances.

As East and Park (1918) have pointed out, the reaction between pollen tube and style must be mutual, otherwise the behavior of self-sterile plants between themselves could not be accounted for. It has been found that substances are produced during the growth of pollen tubes on artificial media that increase the amount and possibly the rate of growth. The tests we have made, however, do not indicate that these particular substances possess the degree of specificity that an explanation of self-sterility on the basis of their activity would demand.

The Formation and Function of Callose Plugs

In the pollen tubes of Gloxinia, Strasburger (1878) first observed that the cavity of the much-elongated vegetative cell is frequently interrupted by short, hyaline structures which he termed *Propfen.* One year later, Elfving (1879) noted the same feature in pollen tubes growing *in vitro.* Numerous subsequent observations indicate their general occurrence. While it was first thought that these plugs are composed of cellulose, Mangin (1890) found that they consist of a closely related substance, callose. Bobilioff-Preisser (1917) reports that in *Vinca minor* the callose plugs may begin to form when the pollen tube is about 1 mm. long and continue to develop at more or less regular intervals of 300 μ as elongation proceeds. When a plug has completely occluded the cavity of the tube, it was observed that other plugs still continue to form in the portion cut off from the tip.

In the comparatively large pollen tubes of *Vinca minor*, the formation of the plugs can be readily observed. In our experiments the pollen was cultured on agar media containing 10 percent cane sugar and a little sterile yeast. At 28° C., germination takes place within 45 minutes. After about 5 hours, plugs may be seen in a few of the longer tubes. As Bobilioff-Preisser (1917) has noted, the plugs arise from around the wall of the tube (figs. 13, 14).[2] As the ring of callose thickens, the passage becomes gradually narrower until, as is shown in figure 15, it is completely closed. Further deposition of callose results in the longitudinal extension of the plug (fig. 16). Bobilioff-Preisser (1917) describes other methods of plug-formation in *Narcissus angustifolius* and *Lathyrus latifolius*, but the end result is the same, the distal portion of the tube becoming shut off from the proximal end. While the first plugs are laid down at more or less regular intervals, our observations agree with those of Bobilioff-Preisser that in the section of the tube cut off from the tip plugs continue to form at random and eventually fill a considerable portion of the cavity. Plug-formation continues throughout the growth period and for some time after the tube has reached its maximum length. In a Vinca tube 4 mm. long, as many as

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[2] The figures referred to in this and the following paragraphs (figs. 13–23) were published in Plate XX, accompanying the third article of this series (in the issue of this Journal for June, 1924).

14 completely formed plugs have been counted; and between some of these, considerable additional callose had been deposited around the walls. The first plug formed usually lies from 400 μ to 700 μ from the pollen grain; at the other end of the tube no plugs have been observed within 200 μ of the tip. After a plug has formed, no further elongation takes place between it and the pollen grain. One tube, kept under observation after the passage had been closed, grew 600 μ during the 3 hours following without altering the distance between the plug and the grain.

Following germination, the food materials stored in the pollen pass into the growing tube (figs. 17, 18). In *Vinca minor* these substances are fats, and their distribution can be readily followed by staining with Sudan III. As the tube elongates, this fatty material is carried forward in the region of the tip (fig. 19), leaving the cytoplasm in the body of the tube clear and translucent as shown in figure 13. It is in this latter region that the callose plugs form. Unfortunately, in Vinca the distribution of the nuclei could not be easily followed. Only two tubes were seen in which they could be distinguished with certainty. One is represented in figure 23 with a single nucleus at the tip; in the other, two nuclei were present and similarly situated. It is a reasonable supposition that in the pollen tubes of *Vinca minor* growing *in vitro* the nuclei lie imbedded in the matrix of fat globules toward the tip.

When a plug is formed, the tip of the pollen tube in which its activity is centered becomes shut off from the parent grain and from the older portion of the tube where elongation has ceased. As growth proceeds, successive plugs are laid down and the size of the active vegetative cell is maintained within fairly constant limits. That the tip of the tube shut off from the parent grain by a callose plug is capable of independent existence was demonstrated by the following experiment: A culture of Vinca pollen about 5 hours old in which plugs were forming freely was placed on the stage of a dissecting microscope. Three tubes were selected, each showing a well formed plug. Each of these pollen tubes was cut in two with fine curved scissors just at the callose plug on the side remote from the tip, and the grain end was discarded. The distance between the plug and the tip on the other piece was measured, and the culture was set away for 1½ hours. At the end of this time the lengths of the tubes were again determined. In two cases, no elongation had taken place; in the third, the isolated tip end of the tube had grown 469 μ. In the first two cases, growth had very probably ceased before the operation was made. To check the matter, a single tube found to be actively growing was similarly treated and the portion between the callose plug and the tip was kept under observation. In the three hours following the removal of the older portion of the tube, the remaining part, closed at the proximal end by the plug, grew 734 μ. If a tube is cut in two in which no plug has been formed or in which the cavity has not become completely closed, the protoplasmic contents ooze out at the severed end and growth ceases at once.

The curve of growth obtained by plotting length of tube against time for Vinca growing on artificial media is somewhat S-shaped (text fig. 2 of the third paper of this series). The first plug is usually formed about the time of most rapid elongation and causes no noticeable change in the shape of the growth curve. During the later stages, as growth slows down and after elongation has ceased entirely, plugs form with greater frequency. Since plug-formation does not alter the rate of growth and often occurs in non-nucleated sections of the tube which, moreover, are quite free from reserve food material, it would appear that this deposition of callose is a secondary phenomenon which accompanies waning activity. Benson (1894) found that the pollen tubes of Carpinus growing in an artificial medium often became plugged just at the junction with the grain and sometimes immediately above the enlarged tip. It is stated that the tip of the tube thus becomes virtually "an isolated spore like a pollen grain, which, though it had lost its outer coat, would in the normal state of things be safely housed or encysted in the tissue of the style." In many woody plants, as described by Coulter and Chamberlain (1915), weeks or even months may elapse between pollination and fertilization. It appears that in these cases the pollen tubes grow into the style and become dormant for a time, resuming growth at a later period. It may well be that in these forms, as Benson (1894) has suggested in the case of Carpinus, callose plugs, forming near the tips as growth declines, serve to isolate them during their quiescent period is a more or less spore-like condition.

Miller (1919) has reported that pollen tubes in maize do not at any time extend the whole length of the silk. Just how general is this dissolution of the older parts of the pollen tube is uncertain. In preparing material for cytological examination by the ordinary methods, the reagents used may remove in large part such vestiges of the tube as remain behind the nucleated tip. In the styles of Nicotiana and Primula killed with hot water, tubes may be traced through the tissues for a considerable distance. We may reasonably suppose, however, that in many long-styled forms the tubes are not continuous from stigma to ovary. From the results of our experiments, showing that the portion of the tube from a fully formed plug forward to the tip is capable of independent and continued growth, it is logical to conclude that in species where the tube does become separated from the grain the formation of callose plugs in series serves to maintain the integrity of the vegetative pollen cell. It should be noted also that by cutting off the older part of the tube the plug limits the region from which the vegetative cell absorbs nutrient materials to the less exhausted portions of the style.

ACKNOWLEDGMENTS

The experimental work reported here was done at the Bussey Institution of Harvard University. The writer wishes to acknowledge his indebtedness to Dr. E. M. East for his helpful criticisms throughout. Credit is due the Department of Genetics of the University of Wisconsin for affording the writer an opportunity to complete the paper while a member of its staff.

Summary and Conclusions

1. A method is described that permits an accurate determination of the rate and amount of pollen-tube growth on artificial media.

2. A very considerable improvement in culture media for pollen was effected through the discovery that sterile yeast added in small amounts to sugar media stimulates growth. In four tests, the pollen tubes of Cucumis showed an average percentage increase in length of 60 percent on the addition of yeast; in six tests with Primula pollen, the growth was similarly increased 72 percent; the addition of yeast to the media in six tests with Lythrum pollen increased the average length of the tubes 142 percent.

3. The growth-promoting substance or substances in the sterile yeast increase the amount but do not alter the type of growth. They are water-soluble, heat-stable, and active in small amounts. It is suggested that they may be protein derivatives or possibly of a vitamine nature.

4. The addition of raw potato juice and extracts of gynoecium parts to artificial media regularly increases the growth of pollen tubes.

5. No evidence was secured to show that nitrogen in the form of inorganic salts promotes growth. Glycocoll and asparagin gave negative results. There was some indication, however, that peptone stimulates growth.

6. It is concluded that pollen tubes may utilize cane sugar, and very probably glucose and fructose, as sources of carbon.

7. The bursting of tubes so frequently encountered in the artificial culture of pollen is interpreted as an osmotic phenomenon.

8. Pollen-tube growth is markedly depressed in the presence of small amounts of various inorganic salts or when sea water is added to the culture medium in concentrations as low as 12 percent. Future studies on the cultural requirements of pollen must be made under laboratory conditions that will permit of an exact control of minute amounts of the various constituents entering into the media.

9. With pollen from Vinca, Scilla, Chionodoxa, Puschkinia, and Muscari, tubes were secured on artificial media as long as, or longer than, the styles of the respective plants.

10. The growth curve of pollen tubes cultured *in vitro* resembles that of an autocatalytic reaction where the amount of substrate is limited.

11. The digestion of the reserve material contributed to the growing tube by the pollen grain was observed and figured. The view is put forward that the form of the curve describing pollen-tube growth *in vitro* is dependent in large part upon the course of the autocatalytic reaction involved in the digestion of these reserve food substances.

12. The difference in form of the growth curves of pollen tubes growing *in vitro* and *in vivo* is best interpreted, we believe, as a result of the difference in the water relations in the two cases. In consequence of the excess of water in artificial media, the amount of synthesis of food material within the tube is almost negligible and the reaction goes almost entirely to the right; in the plant, on the other hand, the amount of water in the system is low and the hydrolytic and synthetic processes proceed at more nearly equal rates, the reserve materials in the tube are replaced from sugars derived from the style, and throughout the cycle of growth no deficiency of substrate is manifested.

13. Anatomical features of the style appear to be sufficient to account for the direction of pollen-tube growth therein. Chemotropism has been demonstrated in the pollen tubes of comparatively few forms. Its importance in determining the direction of pollen-tube growth in the ovarian cavity is problematical.

14. Increases in growth amounting to 34.6 percent and 44.5 percent were obtained on culturing pollen in groups of 4 grains each on yeast and yeast-free media respectively. This increased growth is interpreted as the result of the more complete utilization by the groups, through the proximity of their members, of some diffusible growth-promoting substance or substances, of a catalytic nature perhaps, that are produced by the growing tube.

15. Our experiments have not shown that these particular growth-promoting substances possess the degree of specificity that an explanation of the phenomenon of self-sterility on the basis of their action would demand.

16. It was demonstrated experimentally that the tips of pollen tubes cut off from the older portions by callose plugs are capable of independent growth. Observations were made on the formation of these plugs, and their function in pollen-tube growth is discussed.

17. The distribution of the nuclei in pollen tubes grown artificially was observed in detail and figured. The facts regarding migration and the division of the generative nucleus to form the sperm nuclei indicate close agreement in the behavior of the nuclei in pollen tubes growing *in vitro* and *in vivo*.

18. The literature on the physiology of pollen has been reviewed *in extenso*.

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Reprinted for private circulation from
THE BOTANICAL GAZETTE, Vol. LXXVIII, No. 3, November, 1924

MEIOTIC CYTOKINESIS OF CANNABIS[1]

HUGH C. MCPHEE

(WITH PLATE VII)

During the summer of 1922, the writer investigated the meiotic divisions in the pollen mother cells of *Cannabis sativa*, for the purpose of throwing some light upon the cause of the variability of pollen grain size which occurs in this species. Although the results obtained were not conclusive, so far as the solution of the problems at hand were concerned, it seems desirable to publish a short account of meiosis in this plant, and to add another to the several already reported cases of cytokinesis by furrowing in the cells of higher plants. Since an excellent review of the literature dealing with this interesting phase of plant cytology has been given by FARR (2), it does not seem necessary to take space for literature review here.

STRASBURGER (6) published a few figures of meiotic divisions in *Cannabis*, mostly of the anaphase of the heterotypic division, showing ten chromosomes as the reduced number. In the root tips of the staminate and of the pistillate plants, he found that the chromosomes apparently were identical in size and shape, and no indication of a sex chromosome was observed. The results of recent research, however, have shown that so many irregularities occur in the development of the staminate buds, that a more complete account of chromosome behavior during the reduction division in this plant is desirable. The series of drawings in pl. VII show the changes which take place from the time of the archesporial cell to the liberation of the pollen grains.

Materials and methods

The material for this study consisted of staminate buds fixed whole in ALLEN'S (1) modification of Bouin's fluid. This reagent was found to be very satisfactory for fixing and preserving both the chromatic and the achromatic figures, although there is a slight tendency to cause shrinkage of the tissue. Considerable time and labor were saved by examining one anther from a bud while in the fresh condition, after staining and fixing with aceto-carmine. If the material proved to be in the right stage for study, the remainder of the bud was fixed at once. It was found that the best time to collect buds for studying the reduction division was between 7:00 A.M. and 11:30 A.M. Attempts to obtain satisfactory material in the late afternoon were practically without success.

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[1] Contribution from Bussey Institution of Harvard University.

The examination of large numbers of cells is facilitated by the fact that in a longitudinal section of a staminate bud many pollen mother cells are to be seen within a single loculus. In the premeiotic conditions the archesporial cells are crowded closely together, and occupy the whole space inclosed by the lining of tapetal cells. The greater part of each cell is filled with a rather dense cytoplasm, homogeneous in appearance. In the center or slightly to one side can be seen the large, nearly spherical nucleus, with a well defined membrane, and containing the deeply stained nucleolus. During the premeiotic period the chromatin is in such a fine state of division that no definite units can be observed, and it is not until the formation of chromatin threads during the early stages of synapsis that definite chromatin elements become visible.

The tapetal cells are conspicuous during all stages of meiosis as a ring of closely packed cells, lining the loculus of the anther. During the early synapsis stages of the mother cells, the nuclei of the tapetal cells divide mitotically, but the cells do not divide. These binucleate cells then remain in this condition until the rupture of the mother cell wall, when they begin to disintegrate and soon disappear.

Heterotypic division

The first indication of preparation for the heterotypic division is a slight enlargement of the nucleus and the appearance of a fine network pervading the space inclosed by the nuclear membrane. This fine mesh of threadlike elements appears to extend in all directions in a criss-cross manner, completely filling the nucleus and usually enveloping the nucleolus; but the threads are so fine and cross each other so frequently that it is impossible to make accurate counts of the number present. The next step is a gradual contraction of the chromatin material away from the nuclear membrane.

The condensation is not uniform at all points, and frequently the intertwining mass is found at one side of the nuclear cavity (fig. 4). The continued withdrawal from the periphery of the nuclear cavity causes the individual elements to become very closely massed together. The nucleolus at this time is usually found surrounded by chromatin, although cases have been observed where it was almost free and lay between the chromatin complex and the nuclear membrane. That this period of the nuclear reticulum and chromatin contraction is a relatively long one, is indicated by the fact that much of the material examined was in this stage of development, and seldom did the mother cells at the opposite ends of a loculus show appreciable differences. In some of the later stages, where development is more rapid, it is possible to observe synapsis stages in one end of the loculus and mother cells with two nuclei in the opposite end.

The relatively long period of contraction which passes under the name of synapsis is one during which the chromatin threads are in such close association with one another that little can be learned from observing the closely contracted mass. Fig. 5 shows the appearance of a typical cell at this time. When the chromatin complex begins to expand, the threads spread out in all directions until they reach the limiting membrane. As the threads emerge from the synaptic knot they are thicker, fewer in number, and more twisted about one another than in the early prophase. As the threads reach the membrane they contract more and more, until short, rod-shaped chromosomes are formed. The paired chromosomes which result from this contraction are shown in the region of the nuclear membrane in fig. 8.

From the end of the synaptic period, when the chromosomes are found in the region of the nuclear membrane, to the time when they are arranged in the center of the cell preparatory to being pulled apart to the opposite poles, is a period of many and rapid changes in the cell. The nucleolus, which up to this time has been very conspicuous, begins to fade and finally disappears. At about the same time the nuclear membrane very suddenly disappears. The chromosomes migrate to the center of the cell, the spindle fibers radiate into this mass from the poles of the bipolar cell, and the chromatin units again become indeterminate. The daughter chromosomes remain in this position only a short time before they begin to move apart to the opposite poles of the cell. A polar view of the chromosomes just after they have left the plate shows them well separated and ten in number. The unusual tangential view shown in fig. 12 shows the whole twenty chromosomes as they are leaving the plate, and gives a good demonstration of their morphological identity. As the two groups of daughter chromosomes approach the opposite poles of the cell each begins to contract, and passes through a series of obscure changes, the net result of which is the formation of a complete nucleus. The heterotypic division is now complete, and the binucleate cell awaits the beginning of the homotypic division, which starts almost immediately.

Homotypic division

The homotypic division is characterized by greater rapidity than the heterotypic division, and therefore is less easily observed. At the beginning of this period the chromatin is usually found in a very fine state of division, although it is not uncommon to see small pieces of chromatin which have not undergone complete transformation in the region of the nuclear membrane. The general course of events is much the same as in the heterotypic division, but from the interkinesis condition shown in fig. 14 to the formation of the spindles is an exceedingly obscure period. The threadlike elements of each nucleus contract to form masses of chromosomes, which become arranged in the center of the nuclear cavity; the nuclear membranes and the nucleoli disappear, and spindle fibers appear. The details of these events are difficult to observe. There seems to be much less separation of the chromatin elements during this division than during the heterotypic, and as a result it is practically impossible to follow individual chromosomes. Sometimes both nuclei divide at about the same time, but in other cases one nucleus may be seen in the telophase when the other is just starting to form the mitotic figure. The divisions may both occur in the same plane, or at right angles to each other, or in various planes at different angles to one another. Fig. 15 shows both division figures parallel and in the same plane. The usual result of such an occurrence is that all four nuclei can be seen in one section of the mother cell, a rather unusual condition in *Cannabis*. The most usual condition is to see three of the four nuclei (fig. 17). As the chromosomes leave the equatorial plate in the homotypic division they are much massed together, and only in exceptional preparations can the chromosome number be determined. The final stages which follow are much the same as in the heterotypic division; the four chromosome groups become rounded off, a membrane forms about each, the spindle fibers disappear, and there results a cell with four nuclei. The cytoplasm is homogeneous in appearance throughout the cell, no connecting fibers are to be seen between the nuclei, and traces of a cell plate are entirely absent. The condition of the mother cell at this stage is represented in fig. 17.

Cytokinesis

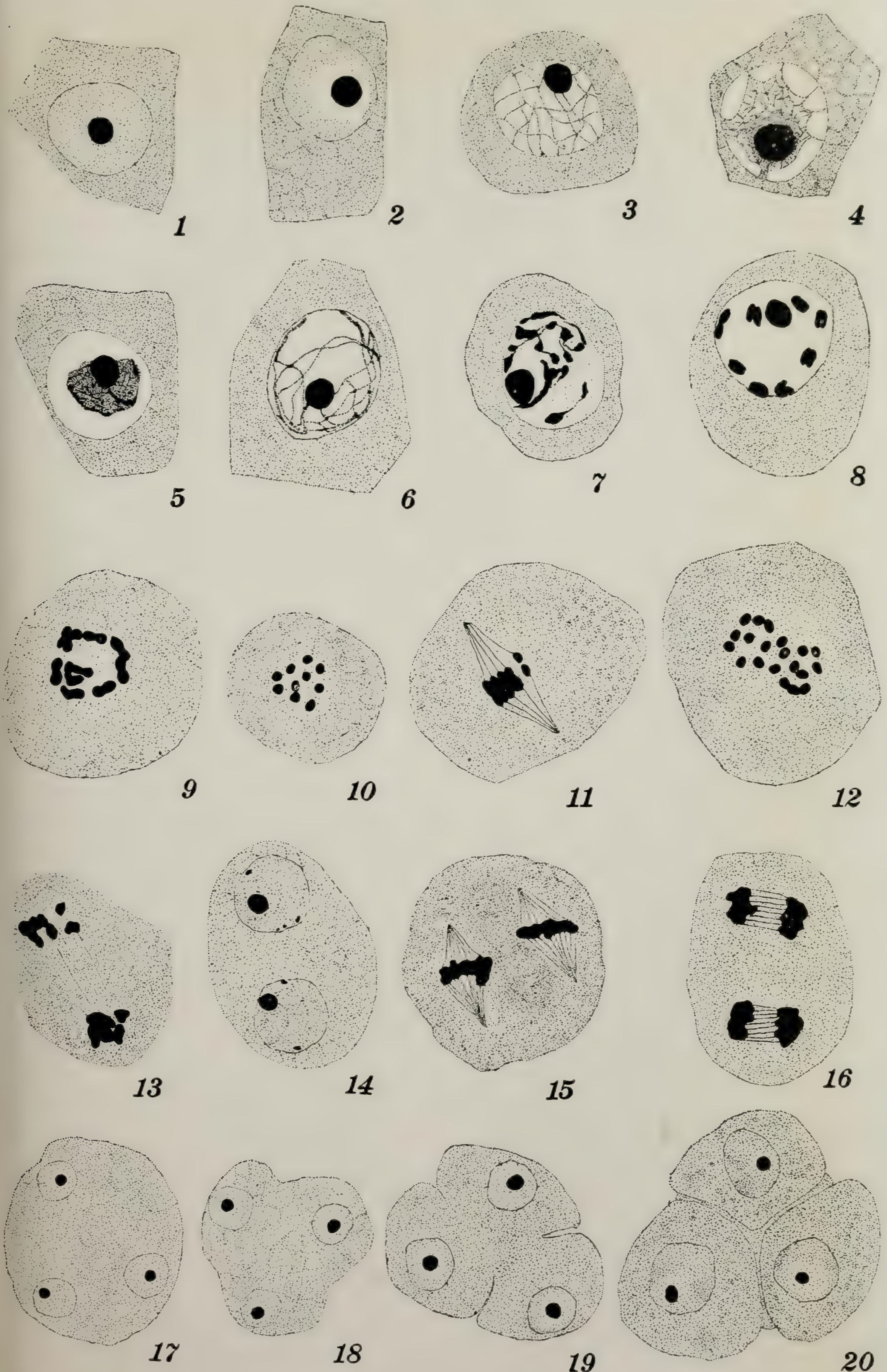
After the meiotic divisions have been completed there is a relatively long period of apparent inactivity within the mother cell. Sections of whole buds frequently show all of the mother cells in this condition, with no indications of further development. The first sign of further change is the appearance of constrictions in the cytoplasm, at four points on the periphery, but inside the mother cell wall and at equal distances from the nuclei. The first visible effect of these constrictions, as seen in a section of the cell, is a change from the nearly spherical to a triangular form. The constrictions become very sharp and extend to the center of the mother cell, cutting it into four parts. Many mother cells can be found which show the completed process, but due to its extreme rapidity, cells which show the constrictions only part way to the center are more rare (fig. 19). The writer has observed a similar process in the mother cells of *Nicotiana*, where it is much less rapid in its development and correspondingly easier to follow.

Until recent years the method of cytokinesis in the dicotyledonous plants was supposed to be by the formation of a cell plate, and this feature was one which constituted a distinction between plant and animal cells. Farr (2, 3), however, has shown that cytokinesis by furrowing occurs in the dicotyledons *Nicotiana*, *Primula*, *Helianthus*, *Ambrosia*, *Tropaeolum*, *Chrysanthemum*, *Magnolia*, and in the monocotyledons *Sisyrinchium* and *Nelumbo*. Mrs. W. K. FARR (4) has found the same process in *Cobaea*. GATES and REES (5) reported a similar process occurring in the pollen mother cells of *Lactuca*. It is evident, therefore, that the occurrence of cytokinesis by furrowing is not as rare among plants as it was thought to be a few years ago, and it is probable that more examples will be found as more plants are investigated. FARR (3) reports that the division of the mother cells in *Nelumbo* is accomplished in part by furrowing and in part by the formation of a cell plate. A cell plate starts to form at the periphery of the cell, and after extending in for a short distance disappears. The process is then completed by the formation of furrows which divide the cell into four parts.

After the formation of the pollen grains as a result of the rapid extension of the furrows to the center of the mother cell, each grows somewhat and rounds off, although the shape of the pollen grains is never exactly spherical while they are retained within the mother cell wall. Finally, the wall of the mother cell is ruptured, the pollen grains pass through a period of growth and assume a spherical form, filling the loculus and awaiting the dehiscence of the anther.

Summary

1. Counts made in the pollen mother cells of *Cannabis sativa* show that the reduced number of chromosomes in this species is ten.
2. The reduction division commonly occurs during the period from sunrise to noon. Although mitotic figures can be found in material collected during the afternoon or evening, they are comparatively rare.
3. The heterotypic division is easily studied, and chromosome counts are most easily made during the anaphase of this division. The behavior of the chromosomes is normal, and no unusual features were noted.
4. Little can be learned concerning the individual chromosomes during the homotypic division, because of the massing of cell elements and the rapidity of the division.
5. There is no morphological evidence that any one of the ten chromosomes is a sex chromosome.



McPHEE on CANNABIS

6. The method of cytokinesis is by furrowing. The furrows appear at the periphery of the cytoplasm and at points equidistant from the mother cell nuclei. These furrows extend rapidly to the center of the mother cell, cutting it into four parts.

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EXPLANATION OF PLATE VII

All drawings were made with the aid of a camera lucida, and all figures were drawn from single sections; magnification 1800 diameters.

Fig. 1.—Archesporial cell.

Fig. 2.—Archesporial cell showing shrinkage of cytoplasm.

Fig. 3.—Parasynapsis.

Fig. 4.—Contraction of chromatin.

Fig. 5.—Synizesis.

Fig. 6.—"Open spireme" showing conjugation of threads.

Fig. 7.—Condensation of threads to form chromosomes.

Fig. 8.—Diakinesis.

Fig. 9.—Metaphase of heterotypic division, polar view.

Fig. 10.—Anaphase, polar view, showing ten chromosomes.

Fig. 11.—Metaphase, side view.

Fig. 12.—Tangential view, late metaphase.

Fig. 13.—Daughter chromosomes at poles.

Fig. 14.—Interkinesis, nuclei in resting condition.

Fig. 15.—Metaphase of homotypic division, side view.

Fig. 16.—Anaphase of homotypic division, side view.

Fig. 17.—Mother cell showing three resting nuclei.

Fig. 18.—Mother cell showing beginning of furrowing.

Fig. 19.—Constrictions extending to center of cell.

Fig. 20.—Four pollen grains before rupture of mother cell wall; mother cell wall not shown.

G-431

THE INFLUENCE OF ENVIRONMENT ON SEX IN HEMP CANNABIS SATIVA L.

BY

HUGH C. McPHEE
(Contribution from Bureau of Animal Industry)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XXVIII, No. 11 : : : Washington, D. C., June 14, 1924

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1925

THE INFLUENCE OF ENVIRONMENT ON SEX IN HEMP, CANNABIS SATIVA L.[1]

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INTRODUCTION

Since environment apparently has such a potent influence on sexual expression in hemp, *Cannabis sativa* L., the writer found it necessary during the course of an investigation of the genetics of sex in this species, to study the effect of relative length of day and night upon vegetative and floral development. The problems connected with this phase of the work have been pursued only as far as was necessary to enable the major work to be carried on and the results are not to be considered as final from the physiological standpoint. But since the work has been discontinued it seems desirable to report the results that they may be available to others who are interested in problems of this kind.

Physiological studies similar to those reported here have been pursued by several investigators, especially Garner and Allard (*1*, *2*, *3*).[2] Since they have reviewed the literature which pertains to this phase of physiology, it seems unnecessary to attempt further review here except to mention Schaffner's work on hemp. Schaffner (*5*, *6*, *7*) found that apparently it is possible to control sex in hemp. When grown during the long days of summer the sexes remain pure and the ratio of staminate to carpellate plants is approximately 1: 1. When grown in the greenhouse during the short days of winter, however, both carpellate and staminate plants showed reversal to the opposite sex. Furthermore, the proportion of plants which showed sex reversal seemed to be roughly proportional to the length of the darkness period. In the shortest days of winter some of the plats showed almost 100 per cent of intersex plants. Schaffner therefore maintains that sex in hemp is non-Mendelian in nature and is under the control of environmental factors.

SEXUAL DIMORPHISM

When grown under normal conditions hemp is when mature dimorphic in both vegetative and flower characters. When grown in the greenhouse during the winter the dimorphism is even more pronounced. The principal sexual differences are as follows:

Staminate plant: More slender and taller than the carpellate plant because of the rapid elongation of the internodes just prior to anthesis; terminal inflorescences with practically no leaves; flowers normally with five sepals and as many anthers; much shorter life than the carpellate type (Pl. 1).

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[1] Received for publication March 26, 1924—issued November, 1924. The results reported in this paper were obtained at the Laboratory of Plant Genetics, Bussey Institution of Harvard University, and completed in the U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 1080.

Journal of Agricultural Research,
Washington, D. C.

Vol. XXVIII, No. 11
June 14, 1924
Key No. G-431

18049—24†

Carpellate plant: More vigorous but shorter than the staminate type; terminal inflorescence leafy; broad crown of leaves; flowers with perianth but no vestiges of stamens; weight at maturity about twice that of the staminate type; longer life.

Probably hemp is as dioecious as any of the so-called dioecious species but it is a well-known fact that certain individual plants sometimes bear both staminate and carpellate flowers at the same time, and that such intersex types appear in larger numbers when the daily exposure to light is short, such as prevails in northern latitudes during the winter months. The vegetative differences, however, are the same for the plants which remain sexually pure and for those which develop some flowers of the opposite sex. That is, a change in the sex of the flowers produced is not accompanied by a corresponding change in the vegetative characters. In other words, the dimorphic vegetative types occur in approximately a 1:1 ratio. It seems quite clear that probably the vegetative type of the plant was already determined in the embryo from which the plant grew but that the sex of each flower was not determined until a somewhat later stage. The natural thing is for each vegetative type to produce flowers of the sex characteristic of that type but so far as any differences which exist in the structural elements of the two types are concerned there is no reason why both sexes of flowers can not be borne by both staminate and carpellate plants. The common occurrence of intersex types in the greenhouse shows that this is true. The writer has several times grafted staminate tops on carpellate stocks and vice versa without any effect upon the sex of the flowers, but does not mean to say that pollen which is produced by a carpellate type plant is the same genetically as that produced by a staminate plant. But since this paper is concerned only with the effect of environmental factors, a discussion of the hereditary aspects of the case will be deferred until a later paper.

PRELIMINARY OBSERVATIONS

The writer's attention was first directed to the effect of relative length of day and night upon sex in hemp by the peculiar behavior of several specimens which were being grown in the greenhouse during the late winter and early spring months. The seed was planted about the first of December and flower buds were first distinguishable on January 4. Relatively few flowers were produced and many of these were so abnormal that few seeds were matured. Following the maturity of this crop of seed the plants remained quiescent for several weeks and then resumed growth during the late spring months flowering again at about the normal blooming time for hemp which is grown in the open. It was thus possible to cause two crops of flowers to be produced in one year. The flowers which were produced during the second flowering period were normal and a fair crop of seed was matured. The same general effects are produced when hemp which has been planted in the open is moved into the greenhouse in the fall. The writer moved several such plants into the greenhouse before killing frosts occurred to ascertain if they could be held over in a semidormant condition. These plants dropped most of their leaves and made no additional growth for several weeks. During the month of December new leaves appeared and a small number of flowers were produced, most of which were abnormal. The production of flowers was still in progress when the plants were discarded late in January. This case refers only to carpellate forms, since the staminate plants die in late summer and apparently can not be kept alive by moving into the greenhouse.

The sexual abnormalities which appear during the short winter days are of the diverse kinds. The most common type among the plats has been the production of intersex flowers on plants of the staminate type. The individual flowers in such cases vary all the way from pure staminate to pure carpellate. The writer has observed flowers containing two anthers and a rudimentary ovulary with one, two, or three stigmas; an apparently normal ovulary and two or three rudimentary anthers; three normal stamens and one terminated with a stigma; two ovularies, each with three stigmas; and many others. Of course, many of such abnormal flowers are not functional and therefore the highly intersex types seldom produce much pollen and set few seeds. Such behavior in the greenhouse during the winter when the daily period of light is relatively short indicates, as Schaffner (*6*) has already shown, that sex in hemp is largely under the control of environmental factors. If this is true it should be possible to obtain almost any desired degree of "sex reversal" by regulating the environmental factors.

PLAN OF THE EXPERIMENTS

For the purpose of producing different lengths of daily exposure to light, a series of ventilated chambers were used from which light could be excluded at will by means of sliding sashes upon a glass roof. The walls of each chamber were covered with black paper to prevent any light which might gain entrance from being reflected. Observation showed that when the shutters were closed these rooms were dark even at noon and gave a sufficiently good imitation of night for the purposes of this experiment. In the discussions which follow these chambers will be spoken of as the dark house. With such an arrangement, the large amount of labor connected with the daily moving of plants in and out of the house was eliminated and the effects of adverse weather conditions, such as high winds and rain, were reduced to a minimum.

The maximum difference in the temperature of the rooms did not exceed 3° C. during the course of the experiment. Although the difference between the temperature of the outside air and that inside the house was much greater than this, such differences are not of importance to this investigation, since adequate control plants were grown in one of the rooms. In work where the control plants were grown in the open it would, of course, be necessary to take into consideration the difference in temperature of the inside and the outside air.

The plants for this experiment were obtained from seed sown in flats in the greenhouse. As soon as the seedlings were well up, the flats were removed to the dark house and the different exposures to light were started at this time and continued until the maturity of the plants. At the time of potting, all of the seedlings were saved.

Inasmuch as soil and size of the pot have a marked effect on plant growth, great care was taken to keep these two factors constant for all lots of seedlings. Sufficient soil was thoroughly mixed at the start to last throughout the experiment. The potting of all lots was always done on the same day so that the plants in each house would have the same chance in this respect. The seedlings were first placed in 2-inch, later in 3-inch, and finally in 5-inch flower pots. To further prevent differences due to nutrition, all pots were placed on wooden benches so that the roots could not go through the bottom of the pots into soil.

Water was applied during the course of the experiment in such quantities as to keep the moisture content of the soil practically the same in all pots. Moisture tests were not made because it was believed that fluctuations in water supply within the limits present in this experiment have no effect upon sex in hemp.

The lengths of exposure used were 5 hours, 7 hours, 10 hours, and the normal length of day for this latitude. By reference to figure 1 it is seen that the period from sunrise to sunset during the course of the experiment was from 12 to slightly over 15 hours daily. The sashes were removed from all compartments at 7 a. m. and replaced as follows: In the 5-hour exposure at 12 m., in the 7-hour exposure at 2 p. m., and in the 10-hour exposure as 5 p. m. Those which were given the full day exposure were treated in the same manner as the other plants except that they were not darkened.

In mentioning sex ratios the number of carpellate to 100 staminate plants has been used throughout this paper.

The effects produced by the various lengths of exposure to light are shown by the summary which is given in Table I.

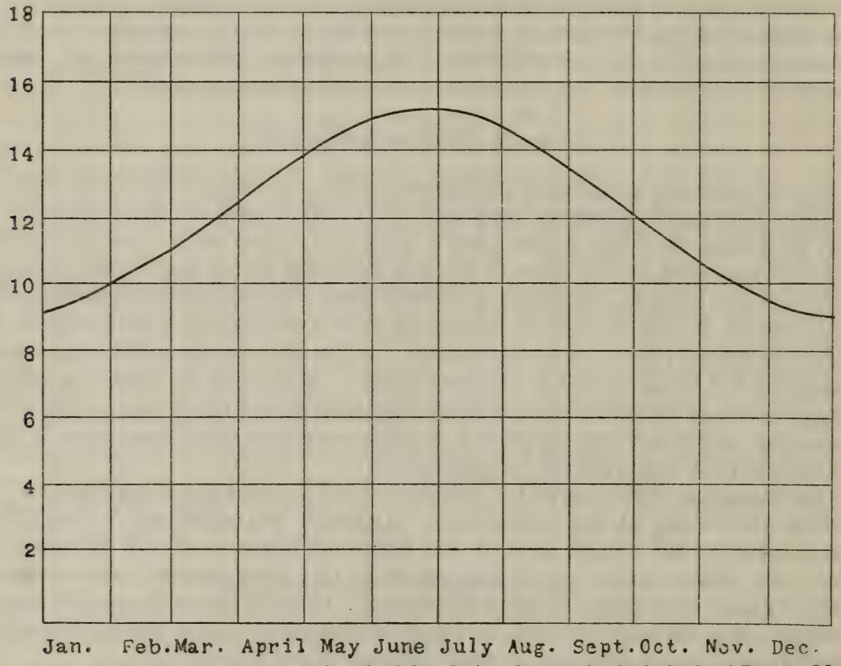

FIG. 1.—Graph showing the changes in length of day during the year in the latitude of Boston, Mass Ordinates indicate 2-hour intervals of the day and abscissae indicate monthly periods of the year

TABLE I.—*Effect of day length on growth and reproduction*

Day length	Date planted	Budded	Sex ratio	Total number	Number died	Height
						Meters
5 hours	May 13	June 21	128:100	128	19	0.21
7 hours	...do	June 14	119:100	101	20	0.50
10 hours	...do	June 15	111:100	95	11	0.75
Day—inside	...do	Aug. 13	115:100	73	23	2.05
Day—outside	...do	July 14	140:100	120	4	1.04
Day—in plat	...do	Aug. 14	97:100	133		3.38
Total			117:100	650	82	

BEHAVIOR OF THE PLANTS

FIVE-HOUR EXPOSURE.—Plants which were exposed to light for only 5 hours daily showed very little development of either the vegetative or the reproductive parts. Side branches were absent and the leaves were few in number, small, and yellowish green in color. The number of flowers produced was very small and the maximum crop of seed matured by single carpellate plants was not over 12. Flower buds first appeared 38 days from the date of planting. The sex ratio was 128:100 in a population of 128. At maturity the mean height of the entire lot was only 0.21 meter and the majority of the plants had to be supported. It is evident that this length of day approaches the lower limit in which hemp can be grown to the flowering stage.

Seven-hour exposure.—An increase of 2 hours daily over the 5-hour exposure not only caused the plants to develop buds 7 days sooner but also caused a significant increase in the development of vegetative and reproductive parts. The mean height attained was 0.50 meter and the stems were sufficiently strong to stand without support. The sex ratio was 119:100.

Ten-hour Exposure.—The chief difference shown by this lot of plants was one of greater development. The mean height at maturity was 0.75 meter, leaves were large and deep green in color, and flowers were very abundant. Buds appeared 33 days from date of planting which is practically the same length of time required by plants in the 7-hour exposure to develop buds. The sex ratio was 111:100.

Full day in dark-house.—The lot which was grown in the dark-house but exposed to the normal length of day for this latitude made so much growth that the size of the pot probably was a limiting factor in their development. The mean height at maturity was 2.05 meters. The main stem did not develop proportionally in thickness but remained slender and many of the plants were unable to support themselves at the time of flowering. The leaves were large and numerous but side branches were not produced to any extent. The time of flowering was greatly delayed in this lot by the long daily exposure to light, buds first appearing 84 days from the date of planting. The sex ratio was 115:100 in a population of 73.

Full day outside in pots.—A number of plants were grown in 5-inch pots in the open as a control on the lot which was grown in the dark-house. Flower buds appeared in 80 days from date of planting. Due to inability to properly control the moisture content of the soil, growth was somewhat irregular and the results obtained with this lot are not comparable with the results obtained with the other lots. The mean height at maturity was 1.04 meters and the sex ratio 140:100 in a population of 120.

Planted in the garden plat.—Hemp which was grown in the garden plat attained a much larger size than did any of the potted plants. The mean height at maturity was 3.38 meters, although individual specimens reached a height of over 4.5 meters and a main stem diameter of over 1 inch. Side branches were produced in large numbers and flower production was very profuse. It is a significant fact that although this lot made a much greater vegetative development, they produced flower buds at the same time as did the lot which was grown in full-day exposure in the dark-house. This result indicates that the differences in development are due to factors other than light and that the time of flowering is not determined by the extent of vegetative development. The sex ratio of the plants in the garden plat was 97:100, the nearest to unity of any of the lots, but of course this is probably only a matter of coincidence.

EFFECT OF LENGTH OF EXPOSURE TO LIGHT ON GROWTH

The results show that both the rate and extent of growth, as well as the time of flowering, are greatly affected by the length of daily exposure to light. The effect on vegetative and sexual development can best be discussed separately.

TABLE II.—*Effect of length of exposure to light on growth*

Exposure	Date planted	Height	
		July 6	Aug. 15
		Meter	*Meters*
5 hours	May 13	0.22	0.47
7 hours	do	.46	.67
10 hours	do	.65	.90
Full day	do	.71	2.28

EFFECT ON VEGETATIVE DEVELOPMENT

Although exact measurements of the details of vegetative development in the dark-house were not taken, the mean heights give a certain measure of the effect of day length on the rate and the extent of growth. Plants in the 5-hour exposure group grew slowly at first which accounts for the small amount of growth made by the time of the first measurements on July 6. The data show that the extent of growth in all lots is roughly proportional to the duration of daily exposure to light. This does not mean, of course, that the rate of growth is proportional to the length of the daily exposure to light. The second series of measurements which were taken on August 15, show the same general effect of day length on height as the first measurements; the plants in the 5-hour day had the lowest mean height and those in the full daylight had the greatest mean height. Although it is realized that such results are not critical, they are very suggestive and agree with the work of Garner and Allard (*3*) who found that the increase in height of soy beans is proportional to the length of daily exposure to light.

Since the length of the growing period was greatly reduced by the accelerating action of the short exposure to light on the reproductive processes in the case of the plants which were grown in a short day, the amount of growth made by these lots was necessarily somewhat limited. The rôles played by the rate and the extent of growth, respectively, is a question which can not be answered from the data obtained in the dark-house. Another carefully controlled experiment has yielded results which show very clearly the relation between rate and extent of growth in hemp. Nine lots of 8 plants each were exposed to light daily for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 7 hours, 12 hours, 18 hours, and 24 hours, respectively. Healthy seedlings of about the same height were selected and the treatment begun at the time of first potting. All seedlings were dark green in color and the mean height of each lot was 5 cm. The growth of each lot is represented graphically in figure 2. The rate of growth is not constant at all times but varies considerably at different periods. All lots began with a fairly low rate which gradually increased but this increase was not the same in all lots and it is due to this differential increase in growth rate that the extent of growth is not the same in all exposures at the end of a certain length of time. The lot which received 24 hours and that which received 18 hours of light, respectively, grew at so nearly the same rate that the data are represented by one curve. Their rate of growth was high from the start and it is due to the rapid growth during the first 10 days of the experiment that the height attained is greater than that of any of the other lots. Plants which were exposed to light for 12 and 7 hours daily were correspondingly slower in attaining a growth rate comparable to that of the 24-hour lot and their mean heights at the end of 34 days was correspondingly less.

Although the extent of growth was greatest in the longer light exposures, it does not necessarily follow that this result is due to a higher rate of growth over the entire period. In fact, the data show that in 7 or 12 hours of light daily the rate of growth at certain times equaled or even exceeded the rate of the 24-hour lot. The greater height at maturity in 18 or in 24 hours of light daily exposure is due to the high rate during the early part of the growth period and the continuance of this rate over a long period of time. If the growth rate which the 7-hour plants have just prior to anthesis could be maintained over a long period they too would be tall but the period of this rapid rate of growth lasts but a few days and then rapidly declines to zero. Since hemp in this exposure grows relatively slowly up to the time of anthesis and then rapidly for only a short time, it necessarily follows that the plants will not attain a great height. The high growth

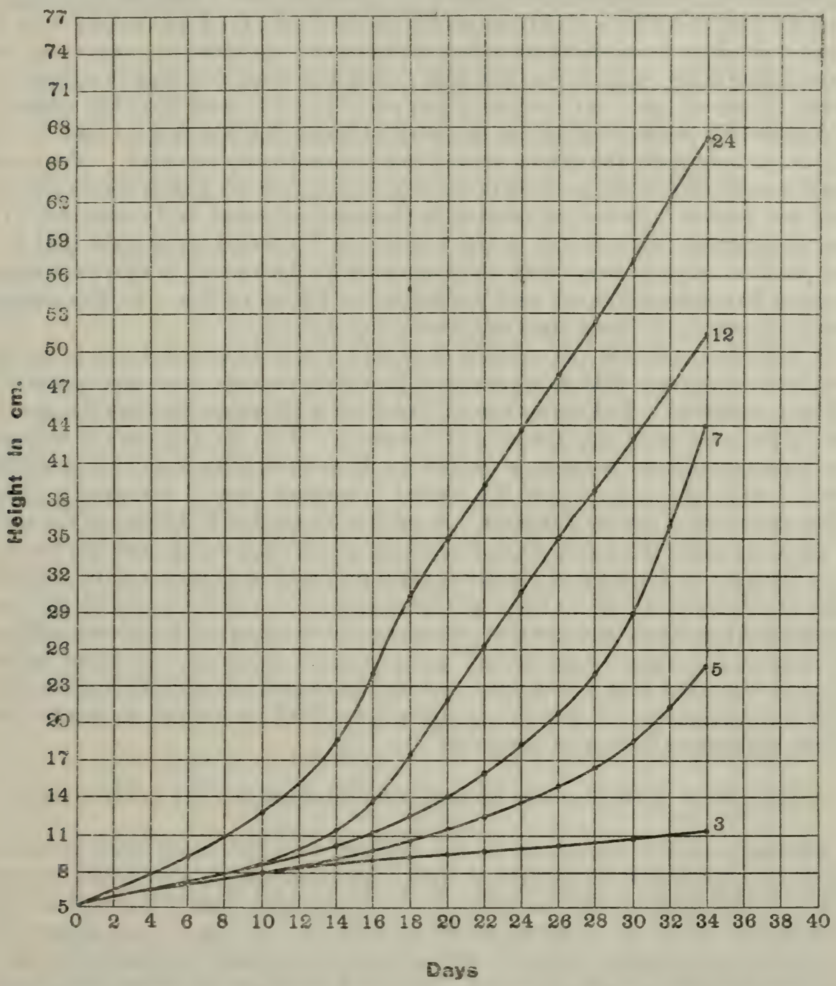

FIG. 2.—Graphs showing relation between relative length of day and night and growth in hemp. The seedlings were 5 cm. in height when the experiment was begun. Ordinates indicate growth in centimeters and the abscissas indicate the number of days. The numbers at the end of each graph show the number of hours of light received daily by each lot of plants. Measurements were made every two days

rate of hemp which is grown in a daily light exposure of 12 or more hours is continued up to the time of flowering, which is about 90 days from planting, and is then increased during anthesis. As a result such plants attain a relatively great height.

The minimum length of daily exposure to light required for continued growth of hemp in this experiment was 3 hours. In an exposure of 1 hour daily the plants were all dead in 20 days, and those which received light for 2 hours daily were all dead in 34 days from the start of the experiment. In these two exposures the growth was fairly rapid during the first 7 days but after this time it rapidly declined to almost zero. At the end of one week from the start these lots began to lose their rich green color and at the time of death the leaves were yellow. In 3 hours of light daily the rate of growth was low and fairly constant. Weakened development was clearly evident in the few, small, greenish yellow leaves and the very few flowers. At the end of 34 days the mean height of the lot was only 11.5 cm. as compared with 44 cm. in the 7-hour lot. It seems quite clear that it is not possible to grow hemp in a daily exposure to light which is short enough to prevent flower development and yet allow the plants to live. In this respect hemp belongs to the same class as Cosmos and Bidens.

It is unfortunate that the analysis of growth in this experiment had to be discontinued at the end of 34 days because it leaves the answers to several interesting points incomplete. The writer has not observed a difference between the growth rate of the sexes until near the time of flowering. With the approach of anthesis the staminate plants increase in height much more rapidly than the carpellate forms. After this point in the life history is reached there is therefore a differential growth rate among the sexes. Since it is impossible to determine the sexes in advance, there is no sure way of selecting equal numbers of each sex in each lot. In order to compare the growth rate from planting to maturity in different light exposures it would be necessary to determine the growth rate of each sex in each light exposure and use this information in working out a correction factor to be applied in cases where the sex ratios were not the same. So in an experiment of this kind with hemp there are two important factors to be considered—a differential growth rate of the sexes, and a second differential rate among the lots due to differences in time of flowering.

EFFECT OF LENGTH OF EXPOSURE ON SEXUAL DEVELOPMENT

Although marked alterations in the vegetative development were produced by various changes in the length of daily exposure to light, equally interesting, and of much greater importance to this investigation, were the effects on the reproductive processes. The length of time between planting and the appearance of flower buds increases as the length of exposure to light is increased from 7 hours daily, and an exposure of less than 7 hours daily also delays the time of blooming. The graphical representation of the data in figure 3 shows the relation between the time of flowering and the length of daily exposure to light. The criterion of time of flowering used here is that time when the sexual nature of the flower buds can be determined with certainty. The actual opening of the flowers does not occur until several days later. In 3 hours of light daily, which is the minimum for growth to maturity in hemp, flower buds appear in about 46 days from date of planting. As the length of daily exposure to light becomes greater the time required for reaching the flowering stage becomes less until an exposure of 7 hours is reached. From this point on, the time between planting and flowering increases rapidly until it is about 90 days in a 15-hour day. Since the experiment in which hemp was exposed to continuous illumination was terminated long before the flowering stage was reached, it is not possible to say whether this lot would have bloomed sooner or later than those which were grown in the normal summer day length. The writer is inclined to believe that there would not be much difference. But with such a curve it is possible to predict the time that buds will appear with considerable accuracy, especially in the shorter exposures. Several times the writer has planted hemp when the period of daily illumination was about 9 hours and has been able to predict within one day of the time when flower buds would appear.

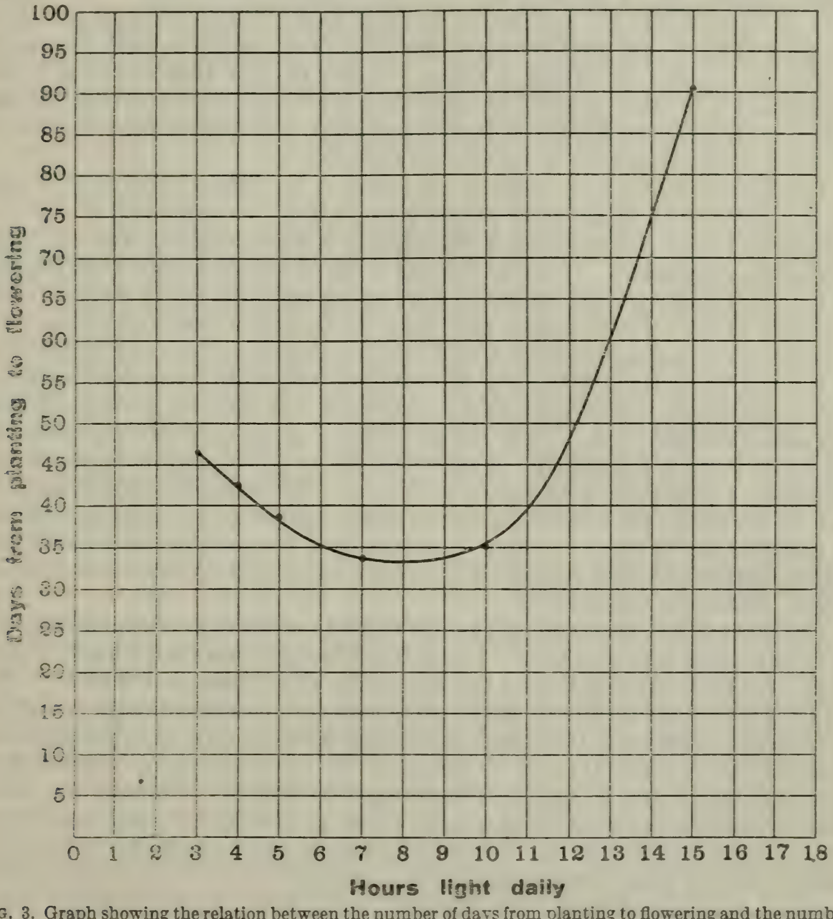

FIG. 3. Graph showing the relation between the number of days from planting to flowering and the number of hours of light received daily. Ordinates indicate 5-day intervals from the time of planting and abscissae indicate the number of hours of light received daily. Hemp can not be grown to the flowering stage in less than 3 hours of light daily

An increase in the length of daily exposure to light after the flower buds appear has been found to greatly affect their further development. In an experiment carried on in the greenhouse during November and December it was found that flower production could be checked by increasing the period of illumination. Three plats were used. Plats two and three were given the normal daylight for that time of the year, but plat one was given, in addition, illumination from a 100-watt electric light from 4 to 8 p. m. every evening. On December 19, flower buds appeared on the plants in plats two and three. At this time the hemp in plat one was still growing vigorously and no flower buds had appeared. Commencing on December 20, plat two was illuminated the same as plat one and in a few days the flower buds which had appeared began to show signs of being stunted. Many of these flowers never opened. All the plants in this plat were stimulated to further growth and no more flowers appeared until the period of illumination was shortened. The use of artificial illumination was discontinued on January 13, and on January 21 the hemp in plat two again showed flower buds. The appearance of buds in plat one occurred at this time also. Thus by merely giving the plants a little longer period of light each day it was possible to delay the time of flowering a month. By giving a longer exposure to light than was given in this experiment it is easily possible to delay blooming even more than this so that hemp can be made to flower in 1 month, 2 months, or 3 months from the time of planting, as the necessities of the case may require.

The differences in the sex ratios of the plants in the different day lengths may be the result of so many other causes that a serious consideration of the discrepancies from the standpoint of the effect of relative length of day and night can not be entertained. It is true that the excess of carpellate plants was greatest in the 5-hour exposure and decreased more or less regularly as the length of daily exposure to light was increased, but when the factors of death and differential germination of seed are considered, such differences cease to have any significance. If it could be assumed that all of the seedlings which died were staminate, the differences in sex ratios could be accounted for, but such an assumption does not seem justifiable here. Since the germination of the seed was only about 65 per cent, there is considerable chance that the excess of carpellate plants was caused by a differential germination of the seed. The sex-ratio differences could also be accounted for in each lot on the assumption of an unconscious selection of seedlings at the time of potting, but since all of the seedlings were saved, this factor does not affect the sex ratio of the total number of plants used in the experiment. This ratio was 117:100.

The effect of relative length of day and night upon flower development was one of extent rather than character. Plants which received less than 7 hours of light daily produced very few flowers but practically all of these were normal. This result is somewhat surprising since the appearance of hermaphrodite flowers on hemp which is grown in the greenhouse during the short days of winter has been reported by Tournois (*8*) and by Schaffner (*5*, *6*, *7*) who have observed that either sex may revert to the other under such conditions. Although the writer has observed similar phenomena in plants grown in the greenhouse, cases of such sexual modifications were very rare among plants grown in short days in the dark house. All the plants were examined very carefully several times but only two cases of such spontaneous "sex reversal" were found among the 650 hemp plants used in the experiment. One of these, and by far the more pronounced of the two, occurred on a staminate type which was growing in the normal length of day for that time of year. This plant produced a few hermaphrodite flowers on one of its branches. The other case was the appearence of a few abnormal staminate buds on a carpellate plant in the 10-hour exposure room. Only a few abnormal anthers were produced and these dried up before any pollen was liberated. These were the only cases of "sex reversal" among plants grown in the dark house and not otherwise treated. It seems quite clear, therefore, that a short exposure to light is not in itself the determining cause of sexual modification in hemp.

THE EFFECT OF MUTILATIONS ON SEXUAL DEVELOPMENT

The two factors which other investigators think are the most important in bringing about a modification of sexual expression in hemp are short daily exposure to light and flower removal. Schaffner (*5*, *6*, *7*) has been able to obtain various degrees of "sex reversal" by growing hemp in the greenhouse during the short days of winter and Pritchard (*4*) has shown that a certain amount of modification of the sexual state is apparently caused by removing the flower buds as soon as they appear. If such factors as length of day and flower removal influence the sexual condition of hemp, it seems reasonable to suppose that the two factors combined might have a decisive influence in causing "sex reversal." To test out this assumption, several plants were selected in each of the light exposures used in the dark house and their flowers were systematically removed during the flowering period.

From 16 staminate plants in the 5-hour exposure a total of 1,832 buds were removed but only two of these plants later developed any carpellate flowers. The hemp in this room was so weak that very few flowers were produced after the operation of flower removal, so that no very definite conclusions can be drawn from this experiment. The carpellate flowers that were produced were very abnormal and occurred in hermaphrodite buds.

From 14 staminate plants in the 7-hour day a total of 5,083 buds were removed. Subsequently, all but one of these plants developed carpellate flowers of various degrees of perfection. Many of these carpellate flowers were abnormal and such forms as a three or four-styled flower, anthers terminated by a style, and mixtures of stamens and carpellate flowers in the same bud, were very common. A certain number of functional carpellate flowers were produced and several seeds were matured.

From six staminate plants in the 10-hour exposure a total of 3,555 buds were removed but only three of these plants later developed any carpellate flowers. A few of these flowers were functional and matured a few seeds. From eight carpellate plants in this day length a total of 260 flowers was removed but only one of the plants so operated on later developed any staminate buds. Since, as already mentioned, one of the carpellate plants in this room developed some staminate buds without having had its flower buds removed, the above cited case of modification of the sexual expression by mutilition does not seem important.

The removal of flowers from hemp which was grown in the open during the long days of summer was without effect in causing modifications of the sexual state. From seven staminate plants a total of 16,425 buds was removed but none of these plants later developed any carpellate flowers. If the removal of the flowers was a determining factor in causing sexual modifications in this species, as Pritchard has suggested, some of these plants should have shown a tendency to develop carpellate flowers but their behavior in this experiment indicates that the mere removal of the flowers will not necessarily cause "sex reversal." In this connection the occasional spontaneous production of normal staminate flowers by carpellate plants in the field should be mentioned. Occasionally carpellate plants of any of the varieties of hemp will produce a few perfectly normal staminate flowers. This is especially true of the Simple Leaf variety. This fact makes the occasional production of a staminate bud by a mutilated carpellate plant entirely without significance and until more positive results are recorded we can conclude that the systematic removal of flowers from hemp which is grown in the field during the long days of summer does not affect the sex of the subsequent flowers.

To obtain more evidence on sexual modification in short days, additional experiments were conducted in the greenhouse during the winter with plants grown from seed matured in the dark-house. The classification of the plants as to sex when they first bloomed showed 164 carpellate : 162 staminate. In a few days after the first flowers opened several hermaphrodite buds were observed, the phenomenon being more common among the staminate individuals. At once the plants were separated into several lots in order to study, in detail, the effect of flower removal during the short days on sexual development.

Early in the course of the experiments it became evident that the material used was sexually different from that used by Pritchard and Schaffner in their work. They state that the tendency is apparently from femaleness to maleness but in the material used in the writer's experiments the tendency appears to be from maleness to femaleness. Only 12.8 per cent of the carpellate plants developed any staminate buds while 53.12 per cent of the staminate plants developed some carpellate flowers. The extent of the change in sex varied greatly in different plants but there can be no doubt that the changes were by far more common among the staminate type.

Although flower removal from staminate hemp plants which are grown in a short daily exposure to light does apparently cause some of them to develop carpellate flowers, there seems to be always a certain number which do not show any sexual changes. For instance, in the above experiment the flower buds were removed from 32 staminate plants in one plat, with the result that 84.3 per cent of them later produced some carpellate flowers. Only 33.3 per cent of those in the control plat showed "sex reversal." In this particular case, then, about 16 per cent of the plants showed no tendency to develop flowers of the opposite sex. Since all of the plants were treated alike, it may be assumed that they are not all genetically identical as regards the sexual state.

A similar experiment on the effect of flower removal on the sexual development of carpellate plants showed less change among the test plants than among the controls. In the plat of 32 plants grown in the greenhouse during the winter, the flower buds were systematically removed as soon as they appeared with the result that 10.53 per cent of them later produced some staminate flowers. In the control plat 14.78 per cent produced some staminate flowers. It is very evident that flower removal from pistillate plants in this experiment has not resulted in significant sexual changes.

Pruning may indirectly have some effect on development but experimental results fail to show any significant modification of sex by this means. When the top of a staminate plant is removed at the time of anthesis, or shortly before, branches arise from the leaf axils and grow to a considerable length. Sometimes varying numbers of hermaphrodite flowers appear on such branches but the results of experiment show that this is probably only a matter of coincidence. In a plat of staminate plants from which the tops had been removed 50 per cent produced some hermaphrodite flowers. In the control plat 50 per cent also showed "sex reversal." In a plat of carpellate plants from which the tops had been removed 18.1 per cent developed hermaphrodite or staminate flowers, while 20 per cent in the control plat produced some staminate flowers. It seems quite clear, therefore, that such drastic pruning as cutting off the whole top of the plant does not significantly affect the sex of the plant.

DISCUSSION

After having observed thousands of hemp plants growing in the greenhouse and in the open, and after having tried many different kinds of stimulation in attempts to change the sex, the writer can not fully agree with the statement that the control of sex in hemp has reached a stage where it is possible to take a sample of seed and produce, at will, a stand of plants of any desired degree of sexual expression. It is true that Schaffner (*6*) has obtained as high as 88 per cent of "sex reversal" in one plat which was grown in the greenhouse during the short days of winter, but when we consider that the plat contained only 16 plants, we must be careful in drawing the conclusion that the same percentage of plants in a much larger plat would show the same changes. Until it is possible to obtain the same degree of sex modification in several plats grown under the same conditions, only tentative conclusions can be drawn concerning the exact control of sexual expression in hemp. It may well be that the genetic balance in different hemp plants is such that a unit change in the environmental factors will produce more visible change in the sex of some than of others. That this balance can be upset and various sexual changes produced by varying the length of daily exposure to light there can be no doubt; but to say that any desired degree of sexual change can be obtained by this means is quite another thing.

The general term "sexual reversal" which has been used by investigators to designate a modification of sexual expression is not entirely a satisfactory one. Pritchard (*4*) and Schaffner (*6*, *7*) have used the term to include all those plants which were first either pure carpellate or pure staminate, but which later produced some flowers of the opposite sex. In the majority of cases these secondary flowers are intersexual and in many cases entirely sterile. Such cases, in the strict sense of the word, are only modifications of the sex of a few flowers. A plant can not be said to have completely reversed its sex until it produces exclusively flowers of the opposite sex. It will be recognized that such a condition is rather rare in any of the so-called dioecious species. Schaffner (*5*, *6*, *7*) has undoubtedly obtained many instances of modification of the sexual expression but not many cases of "sex reversal" in the sense that the plants which were one sex at first produced nothing but flowers of the opposite sex later in their life. A few cases which approach this degree of sex change have been obtained by the writer, however. At least two of the plants which were at first apparently pure staminate, later in their lives produced nothing but carpellate flowers and matured seed. Such cases are as near a complete reversal of sex as probably can be obtained in hemp.

At the present time so little is known concerning the genetic complex of dioecious species of plants that it is not easy to draw conclusions concerning the behavior of the various intersex types. It is known that sex in hemp is somehow influenced by the environment, especially by the relative length of day and night, and also known that the time of flowering of this species is almost entirely controlled, within certain limits, by this same factor. But since the dimorphic vegetative types occur in about equal numbers regardless of the environment there must be something genetic concerned and the fact that one or both of these types later in life produce flowers of the opposite sex, is not necessarily a valid argument that genetic factors are not concerned. Many characters which are genetic in nature are affected by various environmental factors and there is no reason why sex can not be affected likewise. But since the genetics of this particular case is to be published in a later paper, further discussion of the point will not be entered into here.

SUMMARY

1. Hemp possesses a distinct vegetative and sexual dimorphism, but either sex may, under certain conditions, produce flowers of the opposite sex. The vegetative differences are the same for the plants which remain sexually pure and those which do not.

2. The ultimate height attained by hemp appears to be due largely to the length of the period of rapid rate of growth. This period of rapid rate of growth seems to be roughly proportional to the length of daily exposure to light. In a long daily light exposure the high growth rate extends over a long period and the plants are tall at maturity, but in a short daily exposure to light the period

of high growth rate is correspondingly shorter and the plants attain a height of only 1 to 2 feet. The growth rate of hemp grown in a short daily exposure to light may, however, at some time in its life exceed the growth rate of hemp grown in longer daily exposures.

3. The time of flowering of hemp is largely controlled by the relative length of day and night. A daily exposure to light of 7 hours seems to produce the greatest acceleration of the flowering process. A longer or a shorter daily exposure produces a retarding effect on flower development.

4. Three hours of light daily is the least in which hemp will grow for any length of time. If a shorter period of daily illumination is used the plants die before reaching the flowering stage.

5. It is not possible to retard the length of daily period of illumination sufficiently to prevent flowering without causing the death of the plants.

6. "Sex reversal" does not necessarily result when hemp is grown in the greenhouse during the winter months. More intersex types are found under such conditions, but many of the plants remain sexually pure. We may conclude that environment in some way affects the development of sex in this species, but the evidence shows that it does not control it.

7. Removal of flowers does not necessarily result in sexual modifications in hemp, although a few plants so treated and grown in a short daily exposure to light reversed their sex to the extent that some apparently pure staminate individuals matured seed. The occurrence of this phenomenon was not sufficiently general, however, to allow definite conclusions to be drawn. The removal of flowers from hemp which is grown in the field is without effect in causing sexual changes.

8. Although the development and expression of sex in hemp are affected by environmental factors, the changes produced are in many cases relatively minor ones, and a sweeping conclusion that genetic factors are in no way concerned with sex in this species is not warranted at the present time.

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PLATE 1

Right: Staminate hemp plant grown in the greenhouse during the short days of winter. Note clustering of flowers and scarcity of leaves at the top.

Left: Carpellate hemp plant from seed planted at the same time as the plant on the right The clustering of leaves at the tip and the inconspicuousness of the flowers are characteristic of the carpellate type.

A NEW INTERPRETATION OF THE HEREDITARY BEHAVIOR OF SELF-STERILE PLANTS

BY

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Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 11, No. 2, pp. 166–171, February, 1925.

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Communicated December 30, 1924

Among the results appearing from the studies of self-sterility in the genus Nicotiana carried on in this laboratory are the following:

1. Self-sterility is a Mendelian recessive in crosses between self-fertile and self-sterile species. Self-sterile plants, therefore, breed true to self-sterility.

2. Self-sterility in such self-sterile species as *Nicotiana alata* Lk. and Otto and *Nicotiana forgetiana* Hort. (Sand.), or their hybrids, is controlled by numerous inherited factors. Like combinations of these factors give like groups of individuals as far as their compatibility with each other is concerned, just as like factorial combinations which lie at the basis of morphological differences give groups which are alike in those characters.

3. All members of a given self-sterility group are cross-sterile with each other in reciprocal crosses, and all are cross-fertile with members of every other group, with certain limitations as shown in the new data presented in this paper.

4. Not less than fifteen and possibly as high as twenty-five such intra-sterile, inter-fertile groups are found in the material used in these studies.

5. The basis upon which these variations in compatibility rest are hereditarily controlled reactions whereby some pollen tubes (compatible matings) show a rapidly accelerated rate of growth, while other pollen tubes (incompatible matings) show a slow and steady rate of growth which ordinarily does not permit fertilization to occur within the life of the flower.

6. Conditions can be so controlled that (*a*) the length of "life" of the flower is prolonged, (*b*) the rapidity of the growth of the pollen tube is stimulated, and (*c*) the opportunity for pollen-tube action is lengthened.

By taking advantage of various methods mentioned under heading No. 6, chief of which is simply the pollination of the young bud, selfed strains of our self-sterile hybrids between *N. alata and N. forgetiana* have been carried through twelve generations. This lengthy manipulation was carried out with a purpose. If the factors controlling the behavior of these self-sterile plants are distributed according to the regular Mendelian procedure,[1] successive selfings should tend to eliminate heterozygotes. If, furthermore, compatibility between self-sterile plants is brought about by the existence of constitutional differences in certain factors, then one ought to be able to produce near homozygous populations by selfing which would consist of very few intra-sterile, inter-fertile classes.

This expectation has been realized. Numerous families have been raised which consist of two intra-sterile, inter-fertile groups. A smaller number of families have been found to consist of a single group of this type, each individual being cross-sterile with every other individual, though fertile with individuals in other families.

Some twenty-five thousand pollinations have now been made in testing the reactions of this type of family. Data on a few of these families have been published in the sixth paper of these "Studies on Self-sterility"[2] by Dr. Edgar Anderson. We shall call attention first to a peculiarity in these results as follows:

NAME	ORIGIN	CLASSES CROSSED ♀ × ♂	CLASSES RESULTING AND NUMBER OF PLANTS OF EACH	
Family A O	AA–2 × AA–13	a × b	6–b	4–c
Family A L	O–32 × O–46	a × b	8–b	12–c
Family A T	P–27 × O–2	a × b	11–b	6–c
Family A H	L–18 × L–20	a × c	8–b	9–c
Family A I	M–31 × M–25	a × c	9–b	11–c
Family A R	R–38 × L–24	a × c	13–b	7–c
Family A P	R–20 × R–13	c × a	7–a	9–b
Family B P	N–7 × R–12	b × c	6–a	4–c
Family B O	R–2 × O–2	c × b	4–a	6–b

In all of these cases it will be noticed that the class to which the female parent belongs is absent in the progeny. We are dealing with three classes, a, b and c. Each individual of any one of these classes is sterile reciprocally with any other individual of the class to which it belongs and is fertile reciprocally with every individual belonging to either of the other two classes. But the progeny resulting from reciprocal crosses is different. Class "a" × Class "c" gives "b" individuals and "c" individuals only, while Class "c" × Class "a" gives "a" individuals and "b" individuals only. Two classes always appear in what seems to be an approximation to equal numbers, *but the class of the mother is never represented.*

These results have been corroborated numerous times by data obtained since Dr. Anderson's figures were reported, as the following table shows. The classes are here called X, Y and Z, though they prove to be the same classes with which Anderson dealt, when tested by the proper crosses.

NAME	ORIGIN	CLASSES CROSSED ♀ × ♂	CLASSES RESULTING AND NUMBER OF PLANTS OF EACH	
			Class Y	Class Z
Family Q_1 N_1	Q_1 x N_1	Class X × Class Y	21	19
Family R_3 E_2	R_3 x E_2	Class X × Class Y	13	15
Family R_3 R_1	R_3 x R_1	Class X × Class Y	21	13
		Total	55	47
			Class X	Class Z
Family E_2 R_3	E_2 x R_3	Class Y × Class X	21	9
Family O_1 O_2	O_1 x O_2	Class Y × Class X	23	17
Family Q_2 Q_1	Q_2 x Q_1	Class Y × Class X	18	20
		Total	62	46
			Class Y	Class Z
Family O_2 L_1	O_2 x L_1	Class X × Class Z	8	16
Family Q_1 L_1	Q_1 x L_1	Class X × Class Z	21	18
Family R_3 C_{13}	R_3 x C_{13}	Class X × Class Z	20	16
		Total	49	50
			Class X	Class Y
Family L_1 O_2	L_1 x O_2	Class Z × Class X	22	16
Family L_1 Q_1	L_1 x Q_1	Class Z × Class X	8	12
		Total	30	28
			Class X	Class Z
Family N_1 L_1	N_1 x L_1	Class Y × Class Z	11	8
Family Q_2 L_1	Q_2 x L_1	Class Y × Class Z	29	9
Family R_1 C_{13}	R_1 x C_{13}	Class Y × Class Z	12	20
Family R_1 L_1	R_1 x L_1	Class Y × Class Z	16	24
		Total	68	61
			Class X	Class Y
Family R	C_{13} x E_2	Class Z × Class Y	10	10
Family L_1 O_1	L_1 x O_1	Class Z × Class Y	23	13
Family L_1 Q_2	L_1 x Q_2	Class Z × Class Y	23	17
		Total	56	40

It is clear that the same phenomenon is exhibited. When individuals of any two classes are crossed, *two* classes appear in approximately equal numbers, but the class to which the female parent belongs is never represented.

X ♀ × Y ♂	gives	Y and Z	Z ♀ × X ♂	gives	X and Y
Y ♀ × X ♂	gives	X and Z	Y ♀ × Z ♂	gives	X and Z
X ♀ × Z ♂	gives	Y and Z	Z ♀ × Y ♂	gives	X and Y

At first sight one might suppose that these results, differing as they do from any hitherto obtained in genetic work, are due to a novel type of gamete distribution; yet such a supposition is rendered unlikely by two facts. Morphological characters in these varieties appear to be transmitted in the normal Mendelian manner; furthermore the ratios in which the classes appear here are strikingly like those expected when F_1 monohybrids are backcrossed with pure recessives.

To be brief, we believe that the inheritance in these cases is of the ordinary type, but that there are physiological limitations to the opportunity for fertilization.

Assume that in these populations there are three allelomorphs, S_1, S_2 and S_3; and that

Class X = S_1 S_3, Class Y = S_1 S_2, Class Z = S_2 S_3.

Assume further that a plant affords stimulus only to pollen which bears sterility factors other than its own. Thus a Class Z plant (S_2 S_3) affords proper stimulus only to gametes carrying factors other than S_2 or S_3. In other words, of the three factors present in these various populations, the Z plants afford stimulus only to S_1 gametes.

Now what happens when one crosses a Z female by an X male? Only the S_1 pollen is stimulated and functions. The Z female being S_2 S_3 in composition stimulates neither S_2 nor S_3 pollen grains. The resulting progeny consists therefore of two classes, S_1 S_2 (Class Y) and S_1 S_3 (Class X) in equal numbers. In the reciprocal cross, X female (S_1 S_3) by Z male (S_2 S_3), the X female again affords stimulus only to gametes-bearing factors other than its own—this time, the S_2 pollen. The resulting progeny is therefore solely of the two classes Y (S_1 S_2) and Z (S_2 S_3).

The results to be expected according to this hypothesis are in accordance with the data in hand in that (*a*) the class of the female parent is always absent in the progeny, (*b*) the progeny of reciprocal crosses is not the same two classes, and (*c*) the progeny consists of two classes other than the female parent in approximately equal numbers regardless of which of the other two classes serves as the male parent.

This hypothesis can be tested further by selfing individuals or by crossing individuals belonging to the same class. This is done, as explained above, by taking advantage of the "pseudo-fertility" which occurs when non-compatible pollen is given the *opportunity* to effect fertilization. In practice this is usually done by pollinating young buds.

Family R_1 (Class Y = S_1 S_2) selfed produced 32 plants. Of these individuals, 19 were Class Y (S_1 S_2), and showed typical Class Y behavior. Two new classes also appeared. Of the first class, Class Z Y, there were 7 individuals. These plants were fertile as females with all classes except their own, and sterile as males with their own class and also with classes Z and Y. The second class, Class XY of which there were 6 individuals, was fertile as a female with all classes except its own, and was sterile as a male with its own class and also with Classes X and Y.

Similarly Family Q_2 (Class Y = S_1 S_2) selfed gave 24 individuals of Class Y, 14 individuals of Class Z Y and 1 individual of Class X Y.

According to the selective pollen stimulation hypothesis, what happens is this:

Class Y (S_1 S_2), when selfed in the very young bud, gives

$1\ S_1 S_1$ (Class XY) + $2\ S_1 S_2$ (Class Y) + $1\ S_2 S_2$ (Class Z Y).

The S_1 S_1 plants (Class X Y) are fertile as females with

1. Class Z (S_2 S_3) because they accept both S_2 and S_3 pollen,
2. Class X (S_1 S_3) because they accept S_3 pollen, and
3. Class Y (S_1 S_2) because they accept S_2 pollen.

They are fertile as males with Class Z (S_2 S_3) because Z accepts S_1 pollen; but, they are sterile as males with both Class X (S_1 S_3) and Class Y (S_1 S_2), because neither of these classes accept S_1 pollen.

For analogous reasons, individuals of Class Z Y (S_2 S_2) are fertile as females with Classes Z, X and Y, but are sterile as males with Classes Z and Y.

In two other families Y plants were crossed with each other. This should give the same results as when Y's are selfed. The first cross (No. E_2 × No. R_1) gave 17 Class Y + 5 Class Z Y + 1 Class X Y. The second cross (No. R_1 × No. E_2), a reciprocal, gave 21 Class Y + 12 Class Z Y + 6 Class X Y. The total results from Class Y × Class Y(S_1 S_2 × S_1 S_2), therefore are 81 Class Y (S_1 S_2) + 38 Class Z Y (S_1 S_1) + 14 X Y (S_2 S_2). There is a marked deficiency in Class X Y which we are not able yet to ascribe definitely either to differential pollen stimulation or to a lethal effect when S_2 fertilizes S_2. It seems more likely in this particular case to be due to the first cause cited.

There is one bit of evidence on this point. According to the hypothesis, when a Y female (S_1 S_2) is pollinated by an X male (S_1 S_3) only the S_3 pollen should function, resulting in progeny of two classes, viz., Class X (S_1 S_3) and Class Z (S_2 S_3). Such results are normally obtained. But if pollinations are made in the *very young* bud, some of the S_1 pollen might be expected to have the opportunity to function. One family has been obtained from such a pollination, Family R_1 R_3, coming from a pollination of R_1 female of Class Y by R_3 male of Class X. The population obtained consisted of 34 individuals belonging to the following classes, 13 Class X

(S_1 S_3) + 13 Class Z (S_2 S_3) + 4 Class Y (S_1 S_2) + 4 Class X Y (S_1 S_1). According to hypothesis, the cross of S_1 S_2 by S_1 S_3 gave large numbers of Class X (S_1 S_3) and of Class Z (S_2 S_3) because the Class Y female (S_1 S_2) afforded a decided stimulus to S_3 pollen, and gave small numbers of Class Y (S_2 S_1) and Class X Y (S_1 S_1) because very little stimulus was afforded to S_1 pollen. But fertilization with S_1 pollen *was* possible, when the opportunity was forthcoming.

On the other hand, Class Z (S_2 S_3) selfed, which might be expected to give 1 Class Z Y (S_2 S_2) + 2 Class Z (S_2 S_3) + 1 Class Z X (S_2 S_3), excepting as the ratio were disturbed by selective pollen stimulation, did in fact give no plants of Class Z X (S_3 S_3). There were obtained 17 individuals of Class Z (S_2 S_3) and 12 individuals of Class Z Y (S_2 S_2). Likewise, Class X (S_1 S_3), selfed, in two different cases gave 57 individuals of Class X (S_1 S_3) and 20 individuals of Class X Y (S_1 S_1), but not a single individual of Class Z X (S_3 S_3). Since the number of plants in Classes Z Y (S_2 S_2) and X Y (S_1 S_1), respectively, in these two cases showed no particular excess when compared with the heterozygous classes, one must conclude that the heterozygous classes were not composed wholly of S_3 ovules fertilized by S_2 pollen and of S_3 ovules fertilized by S_1 pollen. In each example S_3 pollen must have reached the ovaries and have functioned in the production of heterozygotes. For this reason, it seems as if one were forced to conclude that S_3 is lethal when in the homozygous condition.

[1] Conceivably methods of inheritance which are non-Mendelian might bring about similar results.

[2] The genetic basis of cross-sterility in *Nicotiana*. *Genetics*, **9**, 13–40, 1924.

STUDIES ON SELF-STERILITY. VII. HEREDITY AND SELECTIVE POLLEN-TUBE GROWTH

E. M. EAST AND A. J. MANGELSDORF
Bussey Institution, Harvard University, Forest Hills, Boston, Massachusetts

Reprinted from GENETICS 11: 466-481, September, 1926

GENETICS

A Periodical Record of Investigations Bearing on
Heredity and Variation

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Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879.

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Received March 11, 1926

TABLE OF CONTENTS

	PAGE
INTRODUCTION	466
Presentation of data	469
DISCUSSION	479
SUMMARY	480
LITERATURE CITED	481

INTRODUCTION

A new genetic interpretation of self-sterility as manifested in *Nicotiana alata* Lk. and Otto *var. grandiflora* Comes and *Nicotiana forgetiana* Hort. (Sand.), or their hybrids, has been proposed recently (EAST and MANGELSDORF 1925). The detailed evidence on which this interpretation rests is given in the present paper.

Self-sterility appears to behave as a simple recessive when these species are crossed with the self-fertile species, *Nicotiana Langsdorffii* L. Their interaction is thus similar to that found by COMPTON (1912) in crosses between self-fertile and self-sterile strains of *Reseda odorata* L. The strains of Nicotiana homozygous for self-sterility behave as if their peculiar condition were controlled by several hereditary factors. Like combinations of factors produce like groups of plants in so far as their compatibility with each other is concerned, just as like factorial combinations which lie at the basis of morphological differences produce groups which are alike in those characters. Generally speaking, all members of a given self-sterility group are cross-sterile with each other reciprocally, but are cross-fertile with the members of every other group.

These distinctions in compatibility are due to hereditarily controlled reactions of such a nature that some pollen-tubes (compatible matings) show a rapidly accelerated rate of growth which permits fertilization in from three to five days,—depending mainly on the temperature, while other pollen-tubes (incompatible matings) show a slower and more nearly uniform rate of growth which ordinarily does not allow the generative nucleus to reach the micropyle before the flower withers and falls.

Obviously the differences between compatible and incompatible matings are quantitative, as were the differences between Mendel's tall and dwarf Pisum plants; nevertheless these differences are definite and concrete. There is no more reason for confusing the two types, as certain writers have done, than there is for confusing allelomorphs of numerous well demonstrated heritable differences of the kind commonly termed qualitative. For example, there are species of plants which produce colored flowers under ordinary horticultural conditions; but these plants may be carried through to the flowering stage under conditions which prevent the development of color in the petals, thus they will appear to belong to the same class as plants of the same species which lack the hereditary factor or factors necessary for color development and for which no yet discovered set of environmental conditions can promote color development.

Pollen-tube growth has been studied carefully in both compatible and incompatible matings of these self-sterile Nicotianas. Their growth-curves are characteristically distinct, and under the conditions most frequently met they do not overlap. In every slide studied a few pollen grains do not germinate, or, if they germinate, barely push through the stigmatic tissue. From the pedigree culture results on compatible matings wherein there is no deficiency of expected classes, we feel inclined to believe that this phenomenon is wholly somatic, unconnected with genetic differences. The remaining pollen-tubes in compatible unions show growth-curves having a very narrow variability; that is to say, fertilization in compatible matings is effected between the third and fourth day under all ordinary conditions, and there is probably not more than two hours' difference between the major and minor extremes. Furthermore variations in the environmental factors, such as moisture, plant age, etc., appear to advance or retard pollen-tube growth in these cases but slightly. Here only temperature changes seem to have an influence. In incompatible matings, on the other hand, external conditions have a marked effect. At the height of the flowering season, when pollinations are made on newly opened flowers, the average pollen-tube grows only about 8 mm in the length of time required by the pollen-tubes in compatible matings to traverse the whole length of the style (30 mm to 40 mm). It is noticeable, also, that the dispersion is much greater in the frequency distributions of incompatible matings than it is in compatible matings, though no precise determinations have yet been made. The rate of pollen-tube growth is especially affected by temperature variations and by the relative length of day and night, though it is difficult to determine the exact effect of each factor. One may say with some degree of confidence, however, that with vigorous plants, optimum temperature and light conditions will not affect mid-season incompatible matings sufficiently to produce confused results, though occasionally a small seed-capsule can be obtained.

Fortunately for the analysis of these sterility phenomena, there is another means of obtaining relatively well-filled capsules of seed from incompatible matings. The time during which pollen-tube growth can occur may be materially prolonged. Normally, at mid-season, the individual flower remains on the plant from 6 to 7 days if fertilization does not occur in the meantime. Toward the end of the flowering season this period, the flower life becomes longer. During the last two weeks of flowering the flowers remain alive 9 or 10 days, while one instance is recorded of 14 days.[1] Time of pollen-tube growth can also be extended by bud-pollinations. The old idea that stigmas are only receptive during a limited period, and particularly when they are secreting a substance assumed to promote pollen germination, certainly must have a limited application. In numerous species we have found that pollination of the young bud is fully as efficacious as pollination of the mature flower. In fact it may be that the secretions found in many so-called "ripe" stigmas are merely manifestations of cellular decomposition which occurs after the proper time for pollination. At any rate pollinations can be made 3 days before the flowers open in the species we are considering without any evidence of abnormal effects. In fact pollen-tube growth apparently is faster in bud pollinations than it is in end-season pollinations of open flowers. At least, incompatible matings made on buds 2 days before they open often yield full complements of seed, while similar matings made on newly opened end-season flowers which remain on the plants 3 days over the average, seldom yield more than one-half the normal amount of seed.

By taking advantage of this second means of ensuring fertilization after incompatible matings, inbred strains of our self-sterile hybrids between *N. alata grandiflora* and *N. forgetiana* have been carried through fourteen generations. The purpose of this procedure was to eliminate heterozygotes in case the factors controlling the behavior of self-sterile plants should be distributed in the ordinary manner at maturation. Should compatibility between self-sterile plants be brought about by the existence of constitutional differences in certain factors, then one ought to obtain near-homozygous populations after successive selfings or close matings which would consist of very few intra-sterile, inter-fertile classes. This, in fact, has been the case. Several families have been raised in which the plants all belonged to two intra-sterile groups, while a few families have been found to consist of only one such group. The purpose of this paper is to describe the reactions of these families.

---

[1] SLATE, working in this laboratory, has determined recently that these times may be much greater in certain families.

PRESENTATION OF DATA

As soon as several families were obtained which consisted of but two intra-sterile classes, all possible combinations were made between them. Peculiar but consistent results were obtained. These results can be illustrated by calling the classes X, Y and Z. X crossed by Y gave classes Y and Z in approximately equal numbers. The reciprocal cross Y with X gave classes X and Z in about the same proportions. The same phenomena appeared when the other combinations were made. Class X crossed with class Z gave approximate equality of classes Y and Z, but class Z pollinated with pollen from plants of class X gave equal numbers of classes X and Y. Similarly class Y by class Z produced only X's and Z's in equal numbers, while class Z by class Y produced X's and Y's in equal numbers.

Thus it appeared that when individuals from any two classes were crossed, *two* equal sized classes resulted, but the class to which the female parent belonged was never represented.

These results may be interpreted by assuming that the cross-sterility here shown is controlled by three allelomorphs, S_1, S_2 and S_3; and that class X is S_1S_3, class Y is S_1S_2 and class Z is S_2S_3. If then it is further assumed that a plant affords stimulus only to pollen bearing sterility factors other than its own, all the data are satisfied.

When a Z (S_2S_3) female is crossed with an X (S_1S_3) male only the pollen bearing the factor S_1 is stimulated and functions. Segregation is of the ordinary type, but differential pollen-tube growth causes fertilization to be selective. The resulting progeny, therefore, consists only of the two classes S_1S_2 (Class Y) and S_1S_3 (Class X) in equal numbers. In the reciprocal cross, X (S_1S_3) female by Z (S_2S_3) male, the X female again affords stimulus only to gametes bearing factors other than its own, but this time it is the S_2 pollen. The resulting progeny is therefore solely of the two classes Y (S_1S_2) and Z (S_2S_3). Data from matings of this type are presented in tables 1 and 2.

Six crosses were made where the female parents had the constitution S_2S_3(Z) and the male parents had the constitution S_1S_3(X). Of the resulting plants 405 were classified by crossing with X, Y and Z testers. They fell into two classes; 201 were S_1S_2 while 204 were S_1S_3.

The reciprocal cross, S_1S_3 by S_2S_3, was made seven times. The progeny again fell into two classes, this time S_1S_2 of which there were 233, and S_2S_3 of which there were 205, a total of 438.

TABLE 1

Results of crossing Class S_2S_3 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_1S_3	
L1×8 *L1Q2*	52	59	111
L1×22 *L1Q2*	26	25	51
L1×23 *L1Q2*	39	43	82
L1×7 *R1L1*	56	47	103
L1×*Q1*	12	8	20
L1×*O2*	16	22	38
Total	201	204	405

Most probable expectancy, 202.5 : 202.5 Deviation, 1.5 P. E. 6.8

TABLE 2

Results of crossing Class S_1S_3 female with Class S_2S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
8 *L1Q2*×*L1*	56	50	106
22 *L1Q2*×*L1*	39	40	79
23 *L1Q2*×*L1*	46	46	92
7 *R1L1*×*L1*	43	19	62
Q1 ×*L1*	21	18	39
O2 ×*L1*	8	16	24
R3 ×*C13*	20	16	36
Total	233	205	438

Most probable expectancy, 219 : 219 Deviation, 14 P. E. 7.1

In a second set of experiments, classes Z (S_2S_3) and Y (S_1S_2) were tested. Nine matings were made where S_2S_3 individuals were used as females and S_1S_2 individuals as males. From the resulting population, 493 individuals were tested, of which 248 belonged to the group S_1S_2 and 245 to the group S_1S_3. Ten reciprocal matings were made, S_1S_2 individuals being used as females and S_2S_3 individuals as males. Tests were made on 575 individuals coming from these crosses. Again there were two groups only—312 of class S_1S_3 and 263 of class S_2S_3. Here was found the greatest deviation from equality, a deviation slightly more than 3 times the probable error. The data are set forth in tables 3 and 4.

TABLE 3

Results of crossing Class S_2S_3 female with Class S_1S_2 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_1S_3	
*L*1×10201	44	41	85
*L*1×*E*2	45	40	85
*L*1×1301	26	17	43
*L*1×21*R*1*E*2	23	21	44
*L*1×28*L*1*Q*2	42	58	100
8*R*3*R*1×*R*1	28	12	40
*C*13×*E*2	10	10	20
*L*1×*O*1	13	23	36
*L*1×*Q*2	17	23	40
Total	248	245	493

Most probable expectancy 246.5 : 246.5 Deviation, 1.5 P. E. 7.5

TABLE 4

Results of crossing Class S_1S_2 female with Class S_2S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
10201×*L*1	58	54	112
*E*2×*L*1	39	28	67
1301×*L*1	23	14	37
21*R*1*E*2×*L*1	22	22	44
28*L*1*Q*2×*L*1	56	35	91
*R*1×8*R*3*R*1	25	28	53
*N*1×*L*1	11	8	19
*Q*2×*L*1	50	30	80
*R*1×*C*13	12	20	32
*R*1×*L*1	16	24	40
Total	312	263	575

Most probable expectancy 287.5 : 287.5. Deviation 24.5. P. E. 8.1

In a third series of crosses, plants having constitutions S_1S_2(Y) and S_1S_3(X) were tested. Seven matings were made with S_1S_2 plants as females and S_1S_3 plants as males. From among the progeny, 396 individuals were classified; 189 belonged to group S_1S_3 and 207 to group S_2S_3. Six reciprocal matings were made, S_1S_3 plants being used as females and S_1S_2 plants as males. Testing out 317 of the resulting plants, it was found that 169 belonged to group S_1S_2 and 148 to group S_2S_3. The data for these crosses are found in tables 5 and 6.

TABLE 5

Results of crossing Class S_1S_2 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
*R*1×22 *L*1*Q*2	45	41	86
*R*1×23 *L*1*Q*2	38	51	89
*R*1×39 *R*1*L*1	30	43	73
*R*1×*R*3	14	26	40
*E*2×*R*3	21	9	30
*O*1×*O*2	23	17	40
*Q*2×*Q*1	18	20	38
Total	189	207	396

Most probable expectancy 198 : 198. Deviation, 9. P. E. 6 7

TABLE 6

Results of crossing Class S_1S_3 female with Class S_1S_2 male

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
22 *L*1*Q*2×*R*1	46	45	91
23 *L*1*Q*2×*R*1	40	36	76
39 *R*1*L*1×*R*1	28	20	48
*Q*1×*N*1	21	19	40
*R*3×*E*2	13	15	28
*R*3×*R*1	21	13	34
Total	169	148	317

Most probable expectancy 158.5 : 158.5. Deviation, 10.5 P. E. 6.

Altogether in these six experiments, 2,624 individuals were classified definitely by from one to fifteen pollinations made in various ways with test plants, without a single exception being found. In every cross the progeny fell into two groups of about equal size, and in no case was an individual produced belonging to the class represented by the mother. In addition, about 200 plants were grown which we were unable to classify precisely because unable to make the requisite pollinations. These plants are necessarily omitted from the records. They are reported as a matter of good faith; but it cannot be said that any one of them is an exception, because every plant given *all* of the necessary tests fell definitely into one or the other of the known classes.

Previously published data, recorded by Dr. E. S. ANDERSON, (1924) probably might have been added to the above results for the groups termed

X, Y and Z had we possessed the proper test plants to bridge the gap between his plants and our own. As things stand, all that can be said is that most of ANDERSON's data are also concerned with three classes, and that our plants are descendants of the plants that he used. It is hardly to be questioned that our groups X, Y and Z are his groups A, B and C; but as to which is which we are not certain. Those of ANDERSON's data that can be readily resolved into identical classes consistent with each other are as follows. Three crosses between A females and B males gave 25 B's and 22 C's. Three crosses between A females and C males produced 30 B's and 27 C's. One mating of C female with A male produced 7 A's and 9 B's. One mating between a B female and a C male gave 6 A's and 4 C's. And finally, the reciprocal of this cross, a mating of a C female with a B male, gave 4 A's and 6 B's. Thus in every case the group of the female parent was unrepresented, the progeny belonging to the other two groups, which were represented in approximately equal numbers.

A few other of ANDERSON's figures come from the same family types, but the classes of the parents are unknown. In one case there was an apparent exception which was extremely puzzling. A family R (ANDERSON 1924, p. 32) consisted of 17 individuals belonging to class A and 20 individuals belonging to class C. Family X, which was supposed to have come from the reciprocal of the cross producing family R, gave results identical with those of family R. There were 10 individuals belonging to class A and 5 individuals belonging to class C. This situation was anomalous, for in no other case had reciprocal crosses produced plants belonging to the same two classes. Dr. ANDERSON luckily was able to solve the mystery because he had preserved the original seed envelopes. Several of the crosses used in his work had been made by an investigator who insisted on emphasizing male superiority by writing the male first in the laboratory records. Usually there was no confusion because the male and female signs were used, but in the particular case of family X, the seeming exception, these signs were left off though the figures were in the hand writing of the worker in question. According to the regular custom of this laboratory, therefore, family X was set down as the reciprocal of family R, when it actually was a duplicate of family R, and should have given the similar results that were actually obtained.

The behavior of plants carrying these three self-sterility allelomorphs, S_1, S_2 and S_3, was tested in other ways. Self-pollinations and incompatible cross-pollinations were rendered effective by pollinating buds about three days before they normally would open. Opportunity was thereby given for "like" gametes to fuse.

Family R1 was produced by selfing a plant belonging to class Y (S_1S_2). It consisted of 32 plants, of which 19 were typically class Y (S_1S_2), while 13 belonged to two new classes which were designated ZY and XY. Of the first class, ZY, there were 7 individuals. These plants were fertile as females with all classes except their own, but were sterile as males with classes Z (S_2S_3) and Y (S_1S_2) as well as with their own class. The 6 members of the second class, XY, were fertile as females with all classes except their own, but were sterile as males with class X (S_1S_3) and Y (S_1S_2) as well as with their own class.

Another class Y (S_1S_2) plant selfed, produced 39 individuals (Family Q2), of which 24 proved to belong to class Y, 14 to class ZY and 1 to class XY.

According to the selective pollen stimulation hypothesis class Y (S_1S_2) when selfed in the very young bud yields approximately 1 S_1S_1 (Class XY) + 2 S_1S_2 (Class Y) + 1 S_2S_2 (Class ZY). The S_1S_1 (XY) plants are fertile as females with

(a) Class Z (S_2S_3) because they accept both S_2 and S_3 pollen,
(b) Class X (S_1S_3) because they accept S_3 pollen, and
(c) Class Y (S_1S_2) because they accept S_2 pollen.

On the other hand, they are fertile as males with class Z (S_2S_3) because Z accepts S_1 pollen, but are sterile with both classes X (S_1S_3) and Y (S_1S_2) because neither of these classes accepts S_1 pollen.

Similarly individuals belonging to Class ZY (S_2S_2) are fertile as females with classes X, Y and Z, but are sterile as males with classes Y and Z.

Two other families were produced from this type of mating, but this time two different Y (S_1S_2) plants were crossed with each other reciprocally. The first cross (E2 × R1) yielded 17 class Y plants plus 5 class ZY plants plus 1 class XY plant. The reciprocal cross (R1 × E2) yielded 21 class Y plants plus 12 class ZY plants plus 6 class XY plants. Reciprocal crosses in this case, therefore, gave similar results as might be expected. The collected results from mating S_1S_2 plants together are found in table 7.

The data from these four families, each produced by mating S_1S_2 plants together, show clearly that there is a deficiency of S_1S_1 plants. There were 14 S_1S_1 plants, 81 S_1S_2 plants and 38 S_2S_2 plants. Apparently there is no marked selective fertilization, because there are just about one-half as many S_2S_2 plants as there are S_1S_2 plants. If heterozygotes were formed to the prejudice of homozygotes when there is equal opportunity for fertilization, it would seem as if there should be a great preponderance of S_1S_2 plants. This fact can be explained either by assuming that the S_2

pollen grows slightly faster than the S_1 pollen, or by S_1S_1 homozygotes being weaker than S_2S_2 homozygotes. Both of the homozygous classes are indeed weaker than the heterozygous class; but since they show practically the same vigor from time of germination to maturity, we are inclined to believe that the first postulate is correct. Briefly S_2 pollen grows slightly faster than S_1 pollen. Several additional critical crosses were made involving the homozygous class XY (S_1S_1), the results from which are strictly in accordance with hypothesis.

A homozygous S_1S_1 plant, 9 R1, was selfed in the young bud, giving us Family FA consisting of 31 plants. Each of these plants belonged to the class of the mother (S_1S_1).

TABLE 7

Results from mating Class Y (S_1S_2) plants together.

MATING	RESULT		
	S_1S_1	S_1S_2	S_2S_2
*R*1 (self)	6	19	7
*Q*2 (self)	1	24	14
*E*2×*R*1	1	17	5
*R*1×*E*2	6	21	12
Total	14	81	38

Second, two families were produced by crossing the same plant as above, 9 R1, a homozygous S_1S_1, with a plant O2 belonging to class S_1S_3. Family FB was the result of a normal pollination, Family FC was the result of a pollination made in the young bud. Since by hypothesis the female was S_1S_1 and the male S_1S_3, it is to be expected that only S_3 pollen would function in either case. That is to say, S_3 pollen sufficient to fertilize all the ovules would reach the ovary before any S_1 pollen would arrive. This is what happened. In Family FB the 24 plants tested all proved to belong to the one class S_1S_3, while in Family FC the 32 plants tested all proved to belong to the same class S_1S_3. Thus it seems that selective pollen-tube growth is a strictly individual reaction between the pollen and the style. The growth of a large group of compatible pollen-tubes does not appear to produce anything in the nature of a secretion which *materially* accelerates the growth of incompatible pollen-tubes.

On the other hand, either a *slight* acceleration to the growth of an incompatible pollen-tube may be furnished by the growth of a number of compatible pollen-tubes, or else pollen-tube growth in a *very* young bud may follow a different curve from that followed in the mature flower. For

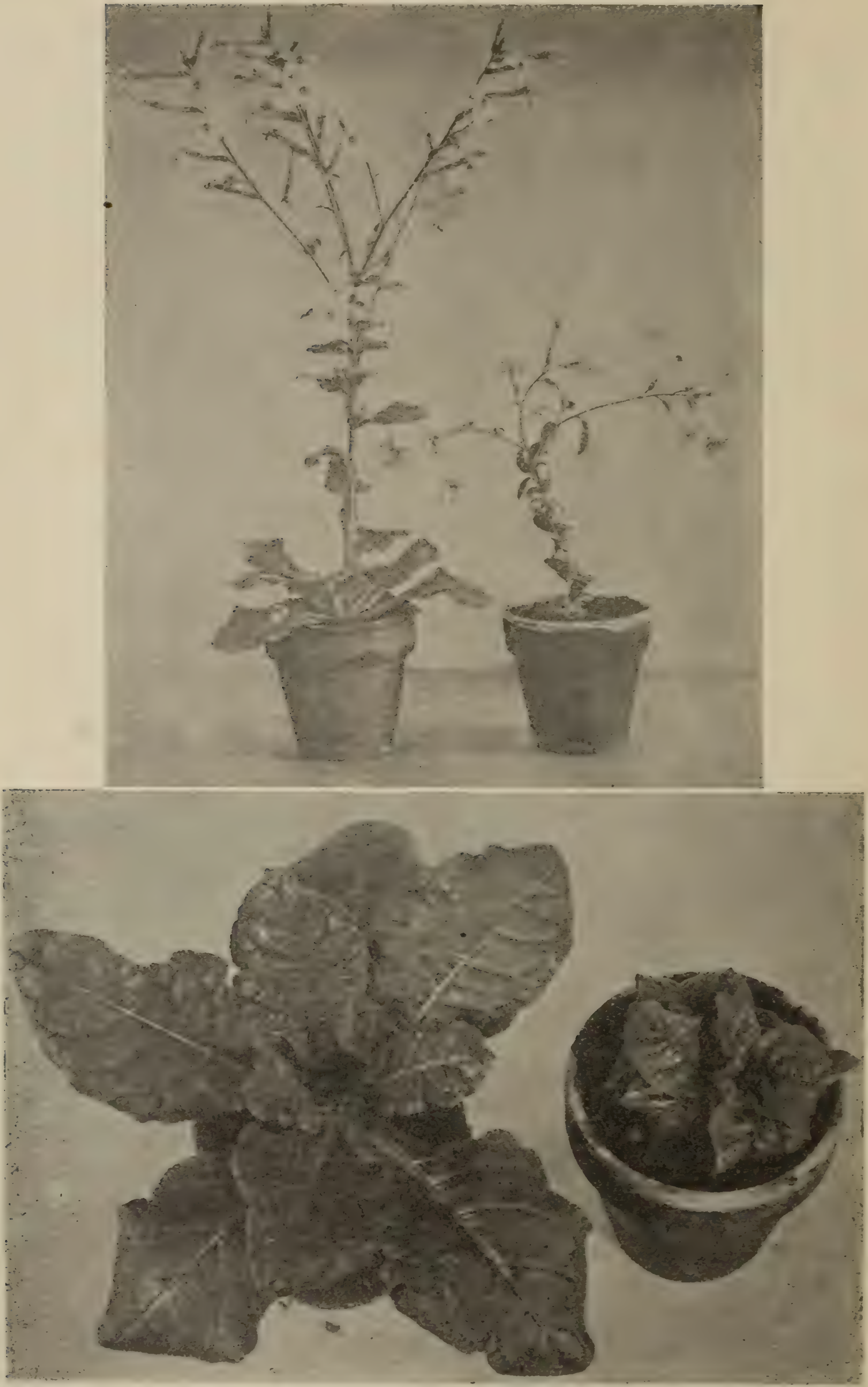

FIGURE 1. Plants of *Nicotiana Sanderae* Hort. showing the effect of the S_3 sterility factor in the homozygous condition. At left, S_1S_3 plants; at right, S_3S_3 plants.

example, normally such a mating as S_1S_2 female by S_1S_3 male gives only the two classes S_1S_3 and S_2S_3. But in one case such a cross made in the *very young* bud, the style being still very short, gave 13 S_1S_3 plus 13 S_2S_3 plus 4 S_1S_2 plus 4 S_1S_1. The same cross made on a mature flower gave 26 S_2S_3 plants and 14 S_1S_3 plants. That this type of phenomenon is not unique is shown further by the results obtained when a S_2S_3 plant (L1) was pollinated in the very young bud stage with pollen from a S_1S_3 plant (O2). The resulting population consisted of 21 S_1S_3 plus 15 S_1S_2 plus 11 S_2S_3 plus 13 S_3S_3. Since in this case an almost equal number of compatible and incompatible pollen-tubes reached the ovary, we are inclined to suggest that the growth-curves of the two types did not have the opportunity to show extreme differentiation because of the relatively short distance traversed. This point is being determined and will be reported upon later.

The final and most critical test of the hypothesis involving S_1S_1 plants was the pollination of two different flowers of the same individual, 9 R1 (S_1S_1), with pollen from plants belonging to classes Y (S_1S_2) and ZY (S_2S_2) respectively. Here are two different crosses, the first being class XY by class Y, the second being class XY by class ZY; yet by hypothesis only S_2 pollen should function in both cases, and all resulting plants should belong to class Y (S_1S_2).

Family FD consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by R1 (S_1S_2). All belong to class Y (S_1S_2). Family FF also consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by 19 Q2(S_2S_2). Again all belonged to class Y (S_1S_2).

Crosses involving the homozygous class ZY (S_2S_2) gave similar results. Plant R1E2 (S_2S_2) when selfed, produced 31 plants all belonging to the one group S_2S_2. Again this homozygous S_2S_2 plant, 4 R1E2, was crossed with pollen from R1 (S_1S_2) producing Family ED, and with pollen from 23 O2 (S_1S_1) producing Family EF. Cross $S_2S_2 \times S_1S_2$ yielded 24 plants all belonging to class S_1S_2, while cross $S_2S_2 \times S_1S_1$ also yielded 24 plants all belonging to class S_1S_2, as was expected.

A smaller series of tests were made with the heterozygous classes X (S_1S_3) and Z (S_2S_3) simply because the apparently lethal character of the homozygous class S_3S_3 diminished the opportunity for producing interesting combinations. But it now happens that the peculiarities of the S_3S_3 group makes it the most interesting of all. For this reason, the experiments with S_1S_3 plants and with S_2S_3 plants will be discussed together.

Two S_1S_3 plants were selfed. The first, O2, produced 30 plants of group S_1S_3 and 13 plants of group S_1S_1; the second, R3, produced 27 plants of group S_1S_3 and 7 plants of group S_1S_1. Altogether 57 plants of class S_1S_3 and 20 plants of class S_1S_1 were obtained.

Likewise, when plant C13 (S_2S_3) was selfed, the resulting population consisted of 17 S_2S_3 plants and 12 S_2S_2 plants.

In none of these populations was a single S_3S_3 plant discovered; yet because there was no marked excess of heterozygotes over the types of homozygotes found, it seemed unlikely that the heterozygotes were produced wholly by the union of S_3 ovules and S_1 or S_2 pollen, as the case might be. It seemed more probable that S_3 pollen had reached the ovaries and had functioned in the production of heterozygotes. For this reason we concluded in our former paper (EAST and MANGELSDORF 1925) that S_3 is lethal when in the homozygous condition.

This conclusion is not wholly correct. In all three of these populations a number of abnormal seedlings appeared, apparently affected by root-rot. They were removed from the seed pans in order not to contaminate the healthy plants. Most of them died at an early age after showing typical root-rot symptoms. A few, however, grew slowly, came to maturity, and proved to belong to class S_3S_3. Every S_3S_3 plant found was of this type. The truth is, therefore, that each of these three populations yielded an unknown percentage of S_3S_3 plants.

While it is not strictly true that S_3 is lethal when in the homozygous condition, it certainly has lethal tendencies. During the winter months, with their short days, it is almost impossible to bring S_3S_3 plants to maturity. When grown during the spring and summer months they are somewhat more viable, but even then they seldom live long enough to produce more than a few flowers. The growth habit is peculiar to themselves. The leaves are more crinkled and the tips more rounded than their normal sisters, and each leaf has a characteristic outward roll, reminding one of spinach. Even fifteen-day-old seedlings can be separated unerringly by their appearance. The flowering branches of the older plants are usually weak and drooping. Efforts to keep plants alive long enough to mature seed capsules have thus far been unsuccessful. The flowers are normal, however, and the pollen in most cases is good. The plants can be classified, therefore, by their reactions as males.

The root-system is always reduced, as if by the action of root-rot. It is barely possible that the whole appearance is the result of a high susceptibility to root-rot, therefore; but the fact that most varieties of plants showing a root-rot infection sufficient to change their whole appearance do not live to maturity, makes it more probable that the morphological changes are due to the direct action of the factor S_3.

It is also possible, of course, that the type comes from the action of another hereditary factor closely linked to S_3, but thus far no crossovers have been found.

Among all of these tests, one can hardly imagine more critical experiments than those made on the homozygous families. Anyone who has puzzled his brain over the peculiar complications emerging from crosses between self-sterile plants will readily appreciate the weirdness of the following results. Here was a family of plants, XY, all inter-sterile. Another family of plants, ZY, was similarly inter-sterile. But XY individuals were fertile with ZY individuals; and reciprocal crosses for the first time produced similar results, for hitherto we had been dealing with matings between heterozygous types where the results of reciprocal matings were unlike. Every plant produced, either by cross XY × ZY or by cross ZY × XY, proved to belong to class Y. That is to say, these F_1 plants all belonged to a single sterility group, and this group was not the same as the two groups to which the parents belonged. Yet when tested as males on the F_1 plants, the two parental types, which had previously been shown to belong to separate classes, might readily have been classified together as one group. In other words, the parental classes XY and ZY were both sterile as males with all plants of their progeny, group Y. On the other hand, XY plants were fertile as females with pollen from Y plants and produced families of all Y plants. Likewise ZY plants were fertile as females with pollen from Y plants and again produced families of all Y plants,—a most astonishing result.

These seemingly anomalous and contradictory results are precisely what is to be expected by hypothesis. Class XY is S_1S_1, class ZY is S_2S_2. These two classes are fertile reciprocally and produce plants all of which belong to the one class $Y = S_1S_2$. Each of these S_1S_2 plants is sterile to the pollen of the parental classes; in the first case because S_1 pollen is not stimulated, and in the second case because S_2 pollen is not stimulated. On the other hand S_1S_1 plants are fertile with the pollen of S_1S_2 plants because S_2 pollen is stimulated and results in S_1S_2 plants being formed, while S_2S_2 plants are fertile with the pollen of S_1S_2 plants because S_1 pollen is stimulated and results in S_1S_2 plants being formed.

DISCUSSION

The analysis of the behavior of the self-sterile Nicotiana plants which has been made in this paper, does not complete even the factorial interpretation for Nicotiana. Another member of the same allelomorphic series, S_4, has been discovered and proved to be distinct from factors S_1, S_2 and S_3.

Three other factors have also been found in material from different sources which may prove to be different from those already described, but critical tests on them have not yet been completed. Nor is it certain that only one locus is involved in the control of self-sterility.

It is hoped that these matters can be more or less definitely settled within the next few months, and that some light can be thrown on the probable number and distribution of factors within these species. But even if matters should thus work out according to our most optimistic expectations, there is still much doubt as to the physiological meaning of the genetic results. It is clear that there is an interaction between the pollen-tubes and the tissues of the styles due to their genetic composition. But it is not certain whether *similar* genetic formulae cause inhibited growth, or whether *different* genetic formulae cause accelerated growth. This distinction may seem like a play on words, but there is a real difference which can be explained only when we know whether more than one locus is involved in controlling self-sterility and what are the exact types of the pollen-tube growth curves.

Some of these problems together with the question as to whether the interpretation of self- and cross-sterility here proposed can be extended to other groups of plants will be discussed in a later paper.

SUMMARY

1. In the populations of Nicotiana used in these studies the behavior and inheritance of self-sterility and of its corollary, cross-sterility, are shown to be determined by three allelomorphic sterility factors S_1, S_2 and S_3.

2. The action of these sterility factors is such that the growth of pollen tubes carrying a given factor is inhibited in the styles of plants carrying that factor.

3. As a result reciprocal crosses between plants having one of the sterility factors in common give unlike progenies, and the sterility group to which the female parent belongs is always absent in the progeny. S_1S_2 ♀ × S_1S_3 ♂ gives equal numbers of groups S_1S_3 and S_2S_3, while the reciprocal cross S_1S_3 ♀ × S_1S_2 ♂ gives equal numbers of groups S_1S_2 and S_2S_3. The other possible combinations give analogous results.

4. Populations produced by selfing in the young bud stage show the expected segregation. Group S_1S_2 selfed gives groups S_1S_1, S_1S_2 and S_2S_2. Group S_1S_3 selfed gives groups S_1S_1, S_1S_3 and S_3S_3. Group S_2S_3 selfed gives groups S_2S_2, S_2S_3 and S_3S_3.

5. Tests of various crosses between both heterozygous and homozygous plants have given the sterility and fertility reactions expected by hypothesis.

6. One of the allelomorphs (S_3) in the homozygous condition produces characteristic abnormalities in habit of growth.

7. New material now being tested has given evidence of additional sterility factors. Complete tests have been made on only one type. It proves to be a fourth allelomorph, S_4, belonging to the same series as those previously described.

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GENETICS, SEPTEMBER 1926

TABLE OF CONTENTS

PAGE

T. A. KIESSELBACH AND N. F. PETERSEN, The segregation of carbohydrates in crosses between waxy and starchy types of maize . . 407

P. C. MANGELSDORF AND D. F. JONES, The expression of Mendelian factors in the gametophyte of maize. 423

HORACE W. FELDMAN, Unit character inheritance of color in the black rat, *Mus Rattus* L. 456

E. M. EAST AND A. J. MANGELSDORF, Studies on self-sterility. VII. Heredity and selective pollen-tube growth. 466

HELEN REDFIELD, The maternal inheritance of a sex-limited lethal effect in *Drosophila Melanogaster*. 482

[This number of GENETICS (11: 407-503) was issued January 24, 1927]

LINKAGE BETWEEN A FLOWER COLOR FACTOR AND SELF-STERILITY FACTORS

BY

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Reprinted from the Proceedings of the National Academy of Sciences,
Vol. 12, No. 4, pp. 248-255 April 1926.

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 12, No. 4. April, 1926.

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Communicated February 17, 1926

Previous investigations concerning the nature and inheritance of self-sterility in plants have shown that the progeny of crosses between two self-sterile individuals fall into a number of definite sterility groups in which each member is sterile with all the other members of the same group, yet is fertile with members of different groups (Cardamine,[1] Linaria,[2] Veronica,[3] Antirrhinum,[4] Nicotiana[5,6,7,8]). Self-sterility and cross-sterility between members of the same sterility group are identical in nature. If pollen from a given plant is placed on the stigma of the same plant or of any other plant belonging to the same sterility group, pollen-tube growth is so slow that the flower ordinarily dies before two-thirds of the distance to the ovary has been traversed. In compatible matings, on the other hand, pollen-tube growth is rapid and fertilization easily accomplished. There is seldom any difficulty in distinguishing between these two cases, yet incompatible matings produce some seed if the pistils are pollinated in the young bud or if, for any reason, flowers persist for a few days longer than is usual.

In certain populations of Nicotiana plants the inheritance of three sterility groups has been investigated in detail.[8] It has been shown that these three groups—X, Y and Z—owe their existence to the action of three allelomorphic sterility factors—S_1, S_2 and S_3. Members of group X are S_1S_3, members of group Y are S_1S_2 and members of group Z are S_2S_3.

The effect of the sterility factors is such that the growth of pollen tubes carrying a given factor is inhibited in the styles of plants carrying this factor.[9] As a result, the flower usually dies before the ovary is reached. Pollen tubes carrying the same factor, however, grow rapidly enough down the styles of plants *lacking* that factor to accomplish fertilization. This interpretation is in agreement with the observation, already mentioned, that self-sterility and cross-sterility are identical in their nature. Thus an S_2S_3 plant, which produces only S_2 and S_3 pollen, is not only self-sterile, but also reciprocally cross-sterile with every other S_2S_3 plant.

A peculiarity in the inheritance of these sterility groups is that reciprocal crosses between members of different groups produce unlike progenies. When, for example, an S_2S_3 plant is pollinated by an S_1S_3 plant, only the S_1 pollen functions, the growth of the S_3 pollen-tubes being inhibited because the female carries S_3. The S_2 and S_3 ovules of the female fertilized by this S_1 pollen give progenies consisting of the groups S_1S_2 and S_1S_3

in approximately equal numbers. If the reciprocal cross is made ($S_1S_3 \times S_2S_3$) the S_1 and S_3 ovules are fertilized by S_2 pollen, the resulting progenies consisting of the groups S_1S_2 and S_2S_3 in approximately equal numbers.

Since reciprocal crosses give different results so far as the transmission of the sterility factors is concerned, it follows that any factor linked with the sterility factors must show corresponding differences. The purpose of the present paper is to give the results of a study of two flower color factors to determine their genetic relations with the sterility factors.

The plants used were members of populations of *Nicotiana Sanderae* (Hort.) = (*N. alata* Lk. and Otto × *N. forgetiana* Hort. (Sand.)), used by East and his co-workers for the determination of the inheritance of the sterility groups.[7,8] Reciprocal crosses between a number of plants were made in the greenhouse during the winter 1924–1925 and the resulting progenies were grown in field plots the following summer. Each plant was crossed with a sufficient number of test plants to determine its sterility group and the flower color of each plant was recorded.

Inheritance of Ivory Flower Color.—The first character to be considered is ivory (*ii*) so-called from the color of the homozygous recessive. The inner surface of the corolla lobes is ivory-colored, while the outer surface is tinged with magenta. The flower color of the heterozygous (*Ii*) and the homozygous dominants (*II*) is non-ivory, the shade of color depending upon the other factors present. The seeds of all three types are brown.

Table 1 shows the inheritance of ivory in three types of crosses. Backcrosses of plants heterozygous for ivory (*Ii*) on ivory (*ii*) give non-ivory and ivory plants in approximately equal numbers. Crosses between plants heterozygous for ivory (*Ii*) give progenies which approximate the expected 3:1 ratio. In both cases there is a small deficiency (about 6%) of the homozygous recessives (*ii*) which may or may not be significant. Progenies from crosses between heterozygous non-ivories (*Ii*) and homozygous non-ivory (*II*) contain no ivories.

The point of especial interest in table 1 is that reciprocal crosses give approximately the same results. Such differences as exist are small and fluctuate in both directions. Since linkage between "*i*" and the sterility allelomorphs must result in differences in the proportions of ivories in the progenies of reciprocal crosses, it is evident that the "*i*" factor and the sterility factors segregate independently of each other.

Inheritance of White Flower Color.—Plants possessing the basic color factor "*C*" show a purplish color at the base of the stem, color in the flowers and brown, rounded seeds. In plants which are recessive for this factor (*cc*) the stem is green, the flowers white and the seeds white and angular.

TABLE 1

INHERITANCE OF IVORY FLOWER COLOR

CROSS	FAMILY	ORIGIN	NON-IVORY	IVORY	PER CENT IVORY
Heterozygous	NA	N1 × 2R1C13	39	28	41.8
Non-Ivory *X*	RA	R1 × 2R1C13	11	12	52.2
Ivory	RD	R1 × 8R3R1	13	11	45.8
		Total	63	51	44.7
Heterozygous	NB	N1 × 7R1L1	43	9	17.3
Non-Ivory *X*	RB	R1 × 7R1L1	41	10	19.6
Heterozygous	NE	N1 × 23L1Q2	46	9	16.4
Non-Ivory	RF	R1 × 23L1Q2	37	7	15.9
		Total	167	35	17.3
Heterozygous	NC	N1 × 8L1Q2	36	0	0
Non-Ivory *X*	RC	R1 × 8L1Q2	54	0	0
Homozygous	ND	N1 × 22L1Q2	55	0	0
Non-Ivory	RE	R1 × 22L1Q2	52	0	0
		Total	197		
Ivory *X*	AN	Reciprocal of NA	33	27	45.0
Heterozygous	AR	Reciprocal of RA	16	12	42.9
Non-Ivory	DR	Reciprocal of RD	14	15	51.7
		Total	63	54	46.2
Heterozygous	BN	Reciprocal of NB	66	15	18.5
Non-Ivory *X*	BR	Reciprocal of RB	66	9	12.0
Heterozygous	EN	Reciprocal of NE	73	19	20.7
Non-Ivory	FR	Reciprocal of RF	59	25	29.8
		Total	264	68	20.5
Homozygous	CN	Reciprocal of NC	90	0	0
Non-Ivory *X*	CR	Reciprocal of RC	92	0	0
Heterozygous	DN	Reciprocal of ND	100	0	0
Non-Ivory	ER	Reciprocal of RE	91	0	0
		Total	373		

Under low temperatures the flowers of the homozygous recessive may show a pinkish tinge, but there is no difficulty in distinguishing between their color and the color of the heterozygous or homozygous dominants. The difference of seed color and shape is constant. White (*cc*) is epistatic to ivory (*ii*).

TABLE 2

INHERITANCE OF WHITE FLOWER COLOR

CROSS	FAMILY	ORIGIN	PROGENY COLORED	PROGENY WHITE	PER CENT WHITE	CROSS	FAMILY	ORIGIN	PROGENY COLORED	PROGENY WHITE	PER CENT COLORED	PER CENT WHITE	DIFFERENCE IN PER CENT WHITE IN RECIPROCAL CROSSES
Colored	LD	L1 × 7R1L1	54	53	49.5	White	DL	Reciprocal of LD	51	19	72.9	27.1	22.4
S_2S_3 X	LE	L1 × 8L1Q2	47	69	59.5	S_1S_3 X	EL	Reciprocal of LE	93	28	76.9	23.1	36.4
White	LH	L1 × 22L1Q2	24	31	56.2	Colored	HL	Reciprocal of LH	67	17	79.8	20.2	36.0
S_1S_3	LJ	L1 × 23L1Q2	50	45	47.2	S_2S_3	JL	Reciprocal of LJ	73	25	74.5	25.5	21.7
		Total	175	198	53.1			Total	284	89	76.1	23.9	29.2
Colored	LB	L1 × 1O2O1	48	48	50.0	White	BL	Reciprocal of LB	37	85	30.2	69.8	19.8
S_2S_3 X	LG	L1 × 28R1E2	22	22	50.0	S_1S_2 X	GL	Reciprocal of LG	13	39	25.0	75.0	25.0
White	LK	L1 × 28L1Q2	48	58	54.6	Colored	KL	Reciprocal of LK	19	77	19.8	80.2	25.6
S_1S_2						S_2S_3							
		Total	118	128	52.0			Total	69	201	25.5	74.5	22.5
Colored	NB	N1 × 7R1L1	52	58	52.7	White	BN	Reciprocal of NB	81	19	81.0	19.0	33.7
S_1S_2 X	NC	N1 × 8L1Q2	37	63	63.0	S_1S_3 X	CN	Reciprocal of NC	90	17	84.1	15.9	47.1
White	ND	N1 × 22L1Q2	55	59	51.6	Colored	DN	Reciprocal of ND	100	20	83.3	16.7	34.9
S_1S_3	NE	N1 × 23L1Q2	57	51	47.2	S_1S_2	EN	Reciprocal of NE	92	14	86.8	13.2	34.0
	RB	R1 × 7R1L1	52	49	48.3		BR	Reciprocal of RB	76	18	80.9	19.1	29.2
	RC	R1 × 8L1Q2	44	40	47.6		CR	Reciprocal of RC	92	9	91.1	8.9	38.7
	RE	R1 × 22L1Q2	52	51	49.5		ER	Reciprocal of RE	91	18	83.5	16.5	33.0
	RF	R1 × 23L1Q2	54	52	49.0		FR	Reciprocal of RF	89	15	85.6	14.4	34.6
	RG	R1 × 39R1L1	47	39	45.2		GR	Reciprocal of RG	67	20	77.0	23.0	22.2
		Total	450	462	50.5			Total	778	150	83.8	16.2	34.3

Three colored plants L1, R1 and N1, each having the constitution *Cc* were crossed reciprocally with a number of white plants (*cc*). The results of these crosses are shown in table 2. Here reciprocal crosses give quite different results. Whenever the heterozygous colored plants L1, R1 and N1 are used as female parents and the homozygous recessive whites are used as male parents—that is, when *C* and *c* ovules are fertilized by *c* pollen, the resulting progenies must consist of white and colored plants in equal numbers. The table shows that this result was obtained. In the total of 1531 plants, 51.5% were white and 48.5% colored (expected 50.0% ± 0.86%).

In the reciprocal crosses where the white plants (*cc*) were used as female parents and the heterozygous colored plants (*Cc*) were used as male parents, equality of colored and white plants can only result if equal numbers of "*C*" and "*c*" pollen tubes accomplish fertilization. It is clear from table 2 that the proportion of colored and white plants in these progenies is far from equal.

These differences in reciprocal crosses might be attributed to a specific effect of the "*C*" and "*c*" factors themselves on the rate of pollen tube growth, if it were not for the fact that in two groups of crosses there is a large excess and in the third an equally large deficiency of colored as compared with white plants. However, if there is linkage between the color factor and the sterility allelomorphs, the results obtained are consistent.

The first group of crosses in table 2 (right half) shows the results obtained when several white flowered plants of the sterility group S_1S_3 were pollinated by the colored L1 plant (S_2S_3). In this cross only the S_2 pollen functions. If the recessive color factor "*c*" is linked with the sterility factor S_2 the offspring should be white (*cc*), except for the cross-overs. If the dominant color factor "*C*" is linked with S_2 the offspring should be colored except for the cross-overs. More than three-fourths of the offspring were colored, indicating that the latter is the case. The constitution of the L1 plant is, therefore ($S_2C\ S_3c$).

This assumption is confirmed by the results of the second group of crosses reported in table 2. Here the same L1 plant is used as the male parent with several white plants of the sterility group S_1S_2. Since in these crosses the S_3c (non-cross-over) pollen and the S_3C (cross-over) pollen functions, the progeny should be predominantly white. Three-fourths of the offspring were white.

In the third group of crosses in the table, the two plants, R1 and N1, both having the constitution S_1S_2, were used as male parents on a number of white S_1S_3 plants. Here only the S_2 pollen functions. There is an excess of colored over white plants in the offspring of these crosses (83.8% colored instead of 50%). It follows that here again the S_2 factor is linked with the dominant color factor "*C*," the white flowered plants being produced by the cross-overs S_2c. The constitution of both the R1 and the N1 plant is, therefore, $S_1c.S_2C$.

Frequency of Crossing-Over between C and the Sterility Factors.—The frequency of crossing-over between the color factor and the sterility allelomorphs in microsporogenesis can be determined from the proportions of white and colored plants in the offspring (table 2, right half). As explained above the cross-overs consist of white flowered plants in the first and third groups and of colored plants in the second group of crosses. The total data are compiled separately for the three plants (table 4). The cross-over percentage in microsporogenesis for L1 is 24.5%, for R1 16.2% and for N1 13.6%.

TABLE 3

LINKAGE BETWEEN WHITE FLOWER COLOR AND THE STERILITY FACTORS IN MEGASPOROGENESIS

PLANT L1

FAMILY	NON-CROSS-OVERS COLORED S_1S_2	WHITE S_1S_3	TOTAL	CROSS-OVERS WHITE S_1S_2	COLORED S_1S_3	TOTAL	PER CENT CROSS-OVERS
LB	39	36	75	5	5	10	11.7
LD	49	43	92	7	4	11	10.7
LE	32	47	79	20	12	32	28.8
LG	17	16	33	6	5	11	25.0
LH	19	21	40	7	4	11	21.6
LJ	35	31	66	4	12	16	19.5
LK	37	49	86	5	9	14	14.0
	228	243	471	54	51	105	18.2

PLANT R1

FAMILY	NON-CROSS-OVERS COLORED S_2S_3	WHITE S_1S_3	TOTAL	CROSS-OVERS WHITE S_2S_3	COLORED S_1S_3	TOTAL	PER CENT CROSS-OVERS
RE	37	38	75	4	7	11	12.8
RF	42	34	76	9	4	13	14.6
RG	35	27	62	8	4	12	16.2
	114	99	213	21	15	36	14.5

For the determination of the crossing-over percentage in megasporogenesis both the color and sterility group for each plant in the progenies must be known. The data for the crosses in which L1 and R1 were used as female parents with several white plants are given in table 3.

In the first group of crosses tabulated in this table the L1 plant ($S_2C.S_3c$) was pollinated with pollen of white flowered $S_1c.S_3c$ and $S_1c.S_2c$ plants. In both cases only the S_1c pollen functions. The L1 plant forms four types of eggs: non-cross-overs S_2C and S_3c, and cross-overs S_2c and S_3C, all of which are fertilized by the S_1c male gametes. In the offspring the colored ($S_2C.S_1c$) and the white ($S_3c.S_1c$) plants are the non-cross-overs and the white ($S_2c.S_1c$) and the colored ($S_3C.S_1c$) plants are the cross-overs.

In the second group of crosses in table 3 the data for the cross between the R1 plant ($S_1c.S_2C$) and several white plants ($S_1c.S_3c$) are given. The non-cross-over eggs of R1—S_1c and S_2C—and the cross-over eggs—S_1C and S_2c—are fertilized by the S_3c male gametes of the white plants used. In the offspring, therefore, the white ($S_1c.S_3c$) plants and the colored ($S_2C.S_3c$) are the non-cross-overs, and the colored ($S_1C.S_3c$) and the white ($S_2c.S_3c$) plants are the cross-overs.

The total data as compiled in table 4 show 18.2% crossing-over for megasporogenesis in the L1 plant and 14.5% in the R1 plant.

TABLE 4

TOTAL CROSSING-OVER PERCENTAGES FOR WHITE FLOWER COLOR AND THE STERILITY FACTORS

NAME OF PLANT	MICROSPOROGENESIS			MEGASPOROGENESIS		
	TOTAL	PER CENT CROSS-OVERS	P. E.	TOTAL	PER CENT CROSS-OVERS	P. E.
L1	643	24.5	±1.15	576	18.2	±1.09
R1	495	16.2	±1.12	249	14.5	±1.51
N1	513	13.6	±1.03			

All data are in agreement with the assumption of linkage between the basic color factor "*C*" and the sterility allelomorphs "*S*." The average crossing-over value approximates 18%. The experiments are not sufficiently extensive to determine whether the differences in the per cent of crossing-over between individuals or between microsporogenesis and megasporogenesis are biologically significant.

Pollen-Tube Growth Factors in Other Plants.—Factors affecting the rate of pollen-tube growth have been postulated in several other cases to explain deviations from expected ratios. (Lychnis,[10] Rumex,[11] Oenothera,[12,13] Zea[14,15]). The situation in these cases apparently differs from that in Nicotiana only in that pollen-tube growth is less strongly inhibited, thus resulting in a deficiency of the class of segregates affected instead of its total absence.[16] It seems quite likely that factors affecting the rate of pollen-tube growth are not uncommon. Any linkage of visible characters with such pollen-tube growth factors must obviously result in disturbed ratios, the degree of disturbance depending upon the closeness of the linkage and the degree of inhibition of the pollen-tube growth. Where aberrant ratios which fail to explain themselves on other grounds are met with in genetical studies, the desirability of reciprocal crosses to test for differential pollen-tube growth is evident.

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THE PHYSIOLOGY OF SELF-STERILITY IN PLANTS.

By E. M. EAST.

[Reprinted from the Jacques Loeb Memorial Volume, The Journal of General Physiology, June 20, 1926, Vol. viii, No. 5, pp. 403–416.]

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THE PHYSIOLOGY OF SELF-STERILITY IN PLANTS.

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(Received for publication, March 22, 1926.)

Hermaphroditic animals and plants are known where fertilization of the eggs by the sperm or by the pollen produced by the same individual is very unusual. The extent of the phenomenon in the animal kingdom is unknown, *Ciona intestinalis*, an ascidian, having been the only species used in experimental work. In the angiosperms, the characteristic is widespread, and must be supposed to have originated many times. At least 50 families are represented, including both monocotyledons and dicotyledons.

The problem which this condition presents is interesting from several angles. The evolutionist wishes to know how and why self-sterility originated, since it often arose in groups where various provisions for cross-fertilization already existed. The geneticist wishes to know whether it is hereditary, and if so what method of inheritance is followed. The physiologist desires an interpretation in physiological terms. And finally, since many apples, pears, peaches, plums, cherries, and other useful fruits are self-sterile, the horticulturist hopes to find a practical method of dealing with the condition in orchard work.

Darwin (5) and his friends and correspondents, Müller (12) and Hildebrand (9), made a number of rather unsystematic cross-pollinations with the plants of several self-sterile species and obtained fruit in each instance. These results led Darwin to conclude that the sexual organs of every such individual have been specialized with respect to every other individual in the same sense in which the external characters of every individual are so specialized that no two are alike. He assumed that differentiation of some sort in the sexual elements is essential to fertilization. After making some 40 or 50 cross-pollinations, he even went so far as to say, "we may therefore confidently assert that a self-sterile plant can be fertilised by the pollen of any one out of a thousand or ten thousand individuals of the same species, but not by its own." This is one of the few cases where Darwin allowed his enthusiasm to outrun his better judgment in issuing generalizations.

Jost (10), the first of twentieth century biologists to attack the problem, came to practically the same conclusion. He was able to show definitely that pollen-tubes from incompatible matings germinate and grow in the styles, but that they grow more slowly than do those from compatible matings. Darwin had suspected that this was the case from observations made on pollen-tubes dissected from living pistils. Jost proved the matter. Jost's hypothesis of individual specialization in the sex elements was the same as that of Darwin; but he recognized a little more clearly that the whole problem was one of differential pollen-tube growth, and he assumed that these differential rates are caused by specific substances which he termed *Individualstoffe.*

These early theories postulating such extreme sexual specialization were rather overdrawn, and were shown to be so by Correns' (4) experiments on the self-sterile crucifer, *Cardamine pratensis.* In this species cross-sterility of the same nature as self-sterility was found. Plants could be grouped into classes, each individual within a class giving the same mating reactions. Thus it was necessary to postulate only a limited number of inhibiting or accelerating agents. Correns endeavored to give a Mendelian interpretation to his results, but failed because of conflicting data. He crossed two plants B and G, and found that the resulting progeny fell into four groups, one group sterile with both parents, one group sterile with the B parent, one group sterile with the G parent, and one group fertile with both parents. His cross-pollinations within this population also show a certain degree of orderliness, but whatever regular order exists is probably masked by the classification as "fertile" of incompatible matings which produce some seed through what I have termed "pseudo-fertility."

In 1913 Compton (3) proved that the genetic difference between self-fertile and self-sterile races of *Reseda odorata* is monofactorial with self-fertility dominant. Self-fertile races are FF or Ff; self-sterile races are ff. That result was corroborated in this laboratory in 1915 (6) by the results obtained from crossing the self-fertile species *Nicotiana Langsdorffii* L. with the self-sterile species *Nicotiana Sanderæ* Hort. and *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, both of these crosses yielding fertile hybrids.

3 years later (8) a microscopical study showed that the pollen-tube growth-curves of compatible and of incompatible matings are different. As shown slightly diagrammatically in Fig. 1, compatible matings produce pollen-tubes whose curves, taken of course by averages, simulate the ordinary two constant autocatalytic curve. They start slowly, but acceleration is rapid, and fertilization usually occurs between the 3rd and 4th day. Presumably if such curves could be followed further, as for example in a delayed pollination of the long styles characteristic of maize, it would be found that retardation would manifest itself later. Incompatible matings, on the other hand produce pollen-tube growth-curves with a very slight acceleration. They approximate a straight line.

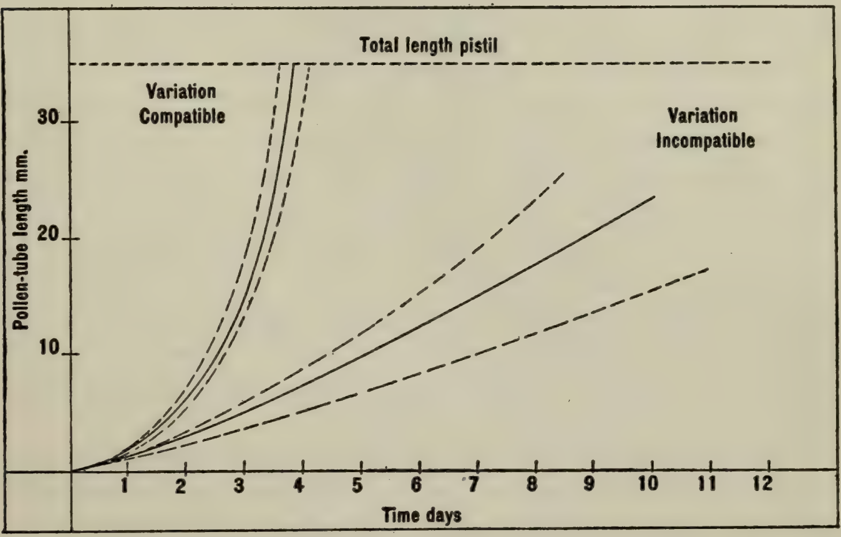

FIG. 1. Diagram illustrating pollen-tube growth-curves in compatible and in incompatible matings, *Nicotiana Sanderæ* Hort.

In compatible matings, though there is considerable dispersion in the frequency distribution of pollen tubes within a single pistil at any given time up to 30 hours after pollination, the pollen-tubes grow so rapidly when nearing the ovary that at any stated time later than 36 hours after pollination the dispersion is very low, probably not more than 2 hours elapsing between the fertilization of the first ovule and the last ovule. In contrast to the narrow variability shown by the growth-curves of compatible pollen-tubes, there is a very great dispersion among the pollen-tube growth-curves within a single pistil after an incompatible mating. At the time when the flower ordinarily withers and falls off, which averages about 8 days, the mean growth is about half the length of the style; but the major and minor extremes may be over a cm. apart. This is quite different from the frequency distribution of the compatible matings when the pollen-tubes are near the ovary. There they are bunched, with probably not over 2 or 3 mm. difference between the major and minor extremes. In passing it may be stated that there appears to be a compensatory growth in long styles. In the *Nicotiana* material the styles vary from 25 mm. to 70 mm., but the proportionate distance of the whole that is traversed in a given time seems to be about the same for styles of various lengths. This observation might perhaps be illusory when dealing with the rapidly growing tubes in late phases of compatible matings; but the same thing occurs in incompatible matings where more precise observations can be made.

Pollen-tube growth in compatible matings is influenced comparatively slightly by external variables such as moisture, fertility of the soil, and length of day, or by such matters as age of plant or time of flowering. Temperature changes have an effect, though they cannot be evaluated quantitatively as yet. One can say only that there appears to be an optimum at about 27°C. On the other hand variations in temperature and in the relative length of night and day have a marked effect on incompatible matings. Furthermore, toward the end of the flowering cycle the flowers often live from 10 to 16 days instead of the usual 7 to 9 days. At this time, with optimum temperature and light conditions, seed may be produced from incompatible matings. This is the occurrence termed *pseudo-fertility*. It is made possible by a correlation between flower persistence and flowerering period rather than age of plant, for a second or even a third flowering cycle reenacts the first. And it is wholly a matter controlled by the flowering cycle, for crosses between two incompatible plants one of which is at the height of the flowering period and the other at the end of the flowering period show pseudo-fertility only when the latter is used as the female parent.

It is clear, then, that self-sterility and self-fertility are two decidedly different conditions, and that these different conditions are hereditary. Nevertheless, since fertilization can occur after an incompatible mating if there is opportunity for gametic union, this difference is quantitative. Furthermore, there are numerous hereditary modifying factors within the species with which we are dealing. The diagram shown in Fig. 2 illustrates the complications that can ensue because of this fact. Strains exist which under ordinary greenhouse conditions show pollen-tube frequency distributions in which the compatible and the incompatible matings yield curves as distinct as those which appear at the extreme right and the extreme left of the diagram. Other strains exist, however, where the frequency distributions from the two types of mating overlap. Fertility becomes confused with pseudo-fertility. In addition confusion can come about from any condition (*a*) which increases the life of the flower and therefore adds to the length of time during which a given pollen-tube has the opportunity to grow, or (*b*) any factor which

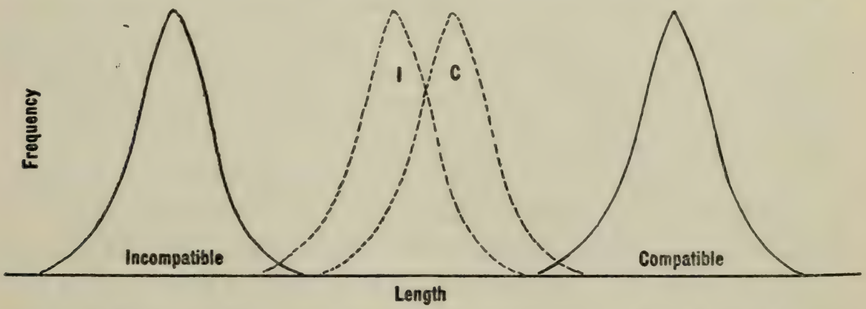

FIG. 2. Diagram illustrating frequency distributions of pollen-tubes after compatible and after incompatible matings at the period when the average of the compatible pollen-tubes is two-thirds the distance to the ovary. Single strains of *Nicotiana Sanderæ* have been found exhibiting curves like the unbroken lines under comparable conditions. Other individual strains under the same conditions exhibit overlapping curves like those shown by the broken lines.

increases the rate of growth of the incompatible pollen-tubes more than it does that of the compatible pollen-tubes.

The proper set-up of the self-sterility problem, therefore, is to select material in which the growth-curves of compatible pollen-tubes are as different as possible from those of the incompatible pollen-tubes, and to keep external conditions constant except when it is desired to obtain seed from incompatible matings.

It is quite evident from an examination of Correns' results on *Cardamine pratensis* that compatible and incompatible matings in this species overlap much as they do in the two broken line curves of Fig. 2. The same statement can be made for the material used so extensively by Stout (13), *Cichorium intybus*. Stout has never appreciated the fact that he should eliminate as many variables as possible from his problems. He has been intrigued by the great complexity of his data. And for this reason he has been unable to show any orderliness in his results.

The first series of cross-pollination studies with the *Nicotiana* species as material was undertaken in 1911. A cross was made between strains of what was thought to be *N. Forgetiana* and *N. alata grandiflora* that had previously bred true to their respective characters. Twenty F_2 plants were crossed in various ways until 154 matings had been made. Excluding selfings and pollinations with the pollen from a plant exhibiting true male sterility, there were 122 cross-matings, of which only three failed and failed repeatedly. While cross-sterility was found, therefore, having apparently the same nature as self-sterility, the frequency was so low that it seemed as if there was almost as much sexual specialization as was predicated by Darwin and Jost. This particular experiment does indeed throw some light on the amount of such specialization, but the conclusions to be drawn from it are more easily understood after a statement is made regarding the results obtained from later experiments carried on with the descendants of this or of similar crosses in which the data are less complex, presumably because of inbreeding.

Over 100 populations from one to fourteen generations removed from this original material have now been tested and retested as to their mating reactions. In every case cross-sterility comparable to self-sterility has been found, and the plants of any given family have fallen into *not more than four* intrasterile, interfertile classes. These results, as shown later, can be interpreted by the hereditary behavior of four mutations of one chromosome locus called S_1, S_2, S_3, and S_4. If self-sterility is controlled by the distribution of genes in one locus only, it is a relatively simple matter genetically; if there are two or more loci involved, things become complicated, whether these loci lie in the same or in different chromosomes. The only evidence that two loci exist comes from the data on the F_2 generation of the original cross, though material from other sources is now being tested, which it is hoped will disentangle the matter.

From a given cross, if but one locus is involved, no more than four intrasterile, interfertile classes can issue. And the size of these classes should be approximately equal. In the original cross described above three sterile matings were found out of 122 cross-matings. If there had been four classes of equal size, and the matings had been at random, 21 per cent of cross-sterility was to have been expected. Disturbance of the equality grouping should result in a rise in the percentage of cross-sterility. In other words, the lowest percentage of cross-sterility to be expected on the assumption that there could not have been more than four intrasterile, interfertile groups is twenty-one except for such slight disturbance as might come about from the fact that certain particular plants (four in number) were mated more often than were the others. The result obtained was approximately 2.5 per cent of cross-sterility. The most probable number of classes to be expected from such a result under the simplest postulates is eight. In addition, it can be shown in two different ways where all the possible matings were made involving certain plants, that six intrasterile groups *must have existed,* unless several errors were made in manipulation or record. This is indirect proof that there are at least two loci controlling the behavior of these self-sterile plants.

Early in the work it was decided to try to simplify genetic analysis by continued inbreeding. This can be done in any manner desired by taking advantage of the phenomenon of pseudo-fertility. Even selfings are usually fruitful if made in the young bud. Should self-sterility (and cross-sterility) be controlled by hereditary factors that segregate and recombine in normal Mendelian fashion, close inbreeding ought sooner or later to result in families where the factorial differences at the basis of self-sterility are reduced to a small number.

Following this method families were soon obtained which were composed of but one or of two classes, each plant within a class being sterile with all other members of that class, and fertile with all members of every other class. A number of two class families characterized by various combinations of the three classes X, Y, and Z have been studied intensively. A class X female mated with a class Y male always gave an approximately equal number of plants belonging to classes Z and Y. A class Y female mated with a class X male, the reciprocal of the above, always gave an approximately equal number of plants belonging to classes X and Z. Other matings gave similar results. Each type of cross produced two classes in nearly equal numbers, and the class of the female parent was never represented.

These odd results are easily interpreted if it is assumed that the active hereditary factors in these matings are S_1, S_2, and S_3, that class X is S_1S_3, class Y is S_1S_2, and class Z is S_2S_3, and that a plant affords a stimulus to pollen-tube growth only when the pollen grain bears a sterility factor other than its own (7).

When a Z female (S_2S_3) is crossed with an X male (S_1S_3) only the pollen bearing the factor S_1 is stimulated and functions. The resulting progeny consist therefore of the two classes S_1S_2 (class Y) and S_1S_3 (class X) in equal numbers. In the reciprocal cross, X female (S_1S_3) by Z male (S_2S_3), the mother plant (S_1S_3) again affords stimulus only to gametes bearing factors other than its own,—this time the S_2 pollen. The result is the production of the two classes Y (S_1S_2) and Z (S_2S_3) in equal numbers.

Practically all possible tests of this hypothesis have now been made and it has been substantiated in every case. By selfing young buds of the heterozygous classes, X, Y, and Z, it has been shown that ordinary Mendelian segregation occurs, and that the anomalous results usually obtained come about only because ordinarily the opportunity for union of like gametes is lacking. When a heterozygous class such as class Y (S_1S_2) is selfed in the very young bud, however, equal opportunity is furnished for all possible combinations of gametes. The resulting progeny consist of plants having approximately the ratio 1 S_1S_1 plus 2 S_1S_2 plus 1 S_2S_2, and these classes show the sterility reactions expected of them. For example, S_1S_1 plants crossed with S_2S_2 plants give only plants of the one class S_1S_2 no matter which way the cross is made. These F_1 plants are sterile with both the parental types, S_1S_1 and S_2S_2, when those types are used as males, because neither S_1 pollen nor S_2 pollen functions normally on S_1S_2 plants. S_1S_2 plants will cross with either parent when used as males, on the other hand, because S_2 pollen functions on the S_1S_1 parent and S_1 pollen functions on the S_2S_2 parent. The result in each case is the production of S_1S_2 plants only.

Thus far four members of this allelomorphic series, S_1, S_2, S_3, and S_4 have been proved to exist. Further, Brieger and Mangelsdorf (2) have shown that each is linked in inheritance with a certain flower color factor and gives a crossover percentage of about 18. This is the first time that hereditary factors having a typical physiological function have been shown to be linked with factors that are primarily morphological in their effect, and shows how arbitrary and illogical such a classification is. It should also be mentioned that S_3, when in the homozygous condition, changes the appearance of the whole plant.

Now what physiological conclusions can be drawn from these results? To discuss this question in its simplest form, it is more convenient to eliminate the effect of variable external conditions on pollen-tube growth and to consider only the effect of heredity in a constant environment. One must admit that such variables as light, temperature, and age of plant are effective, and it is to be hoped that later they can be measured precisely; but this is impossible today.

When thus simplified it is clear that self-sterility, and its corollary cross-sterility, is controlled by a limited number of hereditary factors which function in producing differential pollen-tube growth. Self-sterile plants are not specialized individually in this regard, as Darwin and Jost assumed; but they are specialized as groups. The number of groups is unknown. One allelomorphic series containing four members, has been proved definitely. Three other possibly different members of this same allelomorphic series are now being investigated in order to discover whether they are really distinct from the four already isolated. We will not be certain, however, until many other matings have been completed. In addition, our own early experiments and the experience of Darwin and others wherein an extraordinary amount of fertility was found in crosses made at random, makes it highly probable from a mathematical standpoint that at least one other locus with several allelomorphs exists. Presumably, then, at least two allelomorphic series exist, and the minimum number of different substances affecting pollen-tube growth is six.

The next point to be considered is whether the difference between compatible and incompatible growth-curves is to be interpreted by assuming that *like* factors produce substances that inhibit growth, or by assuming that *unlike* factors produce substances which accelerate growth. The second assumption is more probable. If only one allelomorphic series affecting pollen-tube growth were necessary to account for all data, it would make no difference which interpretation was adopted. With only one active locus, a single mating can give only four intrasterile, interfertile groups. Such a result would be obtained when S_1S_2 plants are mated with S_3S_4 plants. Now it may be that no further complexity need be assumed, since thus far no single mating has given direct proof of more than four of these fertility groups. Yet the earlier experience with material now dead and unusable gives circumstantial evidence of a larger number of groups. Let us postulate a second locus effective in self-sterility and cross-sterility, therefore, which may be called X. If this locus also has four allelomorphs, then a cross between $S_1X_1S_2X_2$ and $S_3X_3S_4X_4$ would give sixteen intrasterile, interfertile classes provided a single factorial *difference* gives the compatible type of growth-curve. If a single factorial *similarity* gives the incompatible type of growth-curve, results would be expected which differ from those thus far obtained. In fact unless a genetic difference promotes pollen-tube growth, no greater complexity is possible than that which yields four intrasterile groups. This conclusion is easily seen to be valid when one examines the results with such crosses as class S_1S_2 by class S_2S_3. If another locus exists which affects pollen-tube growth, both of these groups must be homozygous for it. The complete genetic composition of these classes as regards self-sterility and cross-sterility must be $X_1X_1S_1S_2$ and $X_1X_1S_2S_3$. The weight of the evidence, therefore, is in favor of postulating two series of multiple allelomorphs, though only one has been identified, and of assuming that *any genetic* *difference* in these factors promotes pollen-tube growth of the compatible type.

A second question of some interest is: How do these pollen-tube growth factors act? There is critical evidence on this point. The presence of compatible pollen-tubes in the same style with incompatible pollen-tubes does not change the latter growth-curve materially. Pollen-tube growth being intercellular, it must be assumed, therefore, that the reaction between the stylar tissue and the compatible pollen-tubes is mutual. There is no accelerating agent as such secreted by the cells of the style, called forth by the presence of the compatible pollen-tubes. If this were the case, the incompatible pollen-tubes might be expected to use it and thus grow more rapidly, for experiments on pollen-tubes grown on artificial media indicate that their actual nourishment is of a simple and rather general nature. The indications, therefore, are that easily diffusible substances pass out of the cells of the style and into the membrane of the pollen-tube where they can be manufactured into the necessary growth substances, if the two kinds essential for the final reaction are complementary.

The light shed by these experiments on the evolutionary side of the question is not very dazzling, yet perhaps it may prove to be a serviceable beacon. One has reasonable grounds for several more or less hypothetical conclusions. It is not known for certain whether the factor FF, present in such self-fertile plants as *Nicotiana Langsdorffii*, and in which a change to ff produces self-sterility, is a mutation at the same locus as the allelomorphic series S_1, S_2, S_3, and S_4. If this were the case, then the first mutation from FF to ff (equal to S_1, let us say) would result in a factor that could only be carried along in the heterozygous condition, masked by the fertility factor, and usually under those extraordinary combinations of circumstances which lead to pseudo-fertility. If sterile individuals were formed in rare cases, they would all be cross-sterile with each other, S_1S_1, and would either be eliminated by natural selection or again absorbed into the fertile group by mating with gametes carrying the fertility gene. Perhaps this reasoning can be followed more easily if the factor F is called S_f and its first mutation S_1. The first combination would be the production of fertile heterozygotes, S_fS_1. By analogy with the matings that have been observed, crosses $S_fS_f \times S_fS_1$ and

$S_fS_1 \times S_fS_f$ ought to be possible. The factor S_1 might thus be preserved. But matings S_fS_1 by S_fS_1 might be expected to produce the same results as matings S_fS_1 by S_fS_f, because S_1 pollen-tubes should grow too slowly to compete with S_f pollen-tubes. Actual establishment of a self-sterile strain under this postulate, therefore, would necessarily await a second mutation at this locus giving an additional sterility factor S_2.

What actually happens when the self-fertile species *N. Langsdorffii* is crossed with the self-sterile species *N. Forgetiana* is the production of self-fertile heterozygotes which yield one self-sterile plant to every three self-fertile plants after selfing. Since the factors exhibit no tendency to cause differential pollen-tube growth, one must suppose either that the dominant fertility factor does away with such a tendency, that *N. Langsdorffii* carries other sterility factors, or that the pair of allelomorphs F and f are not of the S series. The latter choice might obtain even if one of the other postulates were true. Experiments are now under way to test these points critically by crossing *N. Langsdorffii* with homozygous S_1, S_2, and S_3 plants. At present there is only one bit of evidence on the subject. Previous experiments have shown that self-steriles extracted from such a cross are of two types showing different reactions. Only one-fourth of the self-steriles, that is to say one-sixteenth of the F_2 population, showed the same degree of self-sterility as was exhibited by the self-sterile grandparent. The remaining three-fourths of the self-steriles appeared to be modified into a decidedly lower grade of self-sterility; and this modifying factor must have been received from the self-fertile grandparent. True, this modifying factor may not be a true self-sterility factor, but it is a factor at a second locus influencing self-sterility. And if one type of self-sterility factor can be carried by a self-fertile plant, it is probable that others can also be carried. The presumption, therefore, is that self-sterility factors can be formed at one or more loci, S or X, in plants homozygous for a fertility factor F, which is wholly independent. If at any time F mutates to f, self-sterile strains can become established immediately, for any tendency of the constantly crossed and recrossed self-sterile plants to show heterosis would influence their survival in competition with the self-fertile plants.

It may be objected that I have here drawn rather far-reaching conclusions from experiments covering only three species, and these from a single genus. There is a certain validity to this criticism, but the situation is not quite so hard as it seems. The experiments of Stout (13) on *Cichorium intybus*, of Correns (4) on *Cardamine pratensis*, of Lehmann (11) on *Veronica syriaca* and of Baur (1) on various Spanish *Antirrhinums* show a regularity of the *type* which this hypothesis demands. The species used by Stout and by Correns are so pseudo-fertile that a critical reanalysis of their results is impossible, but no data were obtained that are directly opposed to the demands. Lehmann, who had better material, did not make the matings needed to determine the points at issue. Baur, on the other hand, made the crosses essential for an evaluation according to the scheme described here, using a species he called *Antirrhinum hispanicum*. (In a recent letter, he calls this material *A. Segovii N.S.*) He obtained two intra-sterile, interfertile groups as the result of crossing two plants E II and E V. Yet as he did not give the exact parentage of the members of the F_1 generation in his published paper, his work could not be reanalyzed. E II was fertile with both classes of the progeny. E V was sterile with all of the plants of one of the classes. If then E II were the female parent belonging to class S_1S_2 and E V were the male parent belonging to class S_1S_3 (his class "a") then the cross should have produced equal numbers of S_2S_3 (a new class fertile with both parents) and of S_1S_3 (his class "a," the assumed class of the male parent and sterile with it). What he actually obtained was 13 plants of class "a" (S_1S_3), the same as the assumed class of the male parent; and 13 plants of class "b", a class different from either parent. In other words, if his cross were E II female by E V male, the results corroborate the hypothesis described in this paper; if E V were the female and E II the male in the cross, the results are antagonistic to the hypothesis. In his letter of February 19, 1926, Professor Baur states that the cross was E II female by E V male. Thus there is complete corroboration of the hypothesis on plants belonging to the family *Scrophulariaceæ*, a family which, while closely related to the family *Solanaceæ*, is sufficiently removed from it to make it improbable that the same mechanism would control self-sterility in both cases if it were not a rather general type.

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WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

[Reprinted from PSYCHE, Vol. XXXIII, No. 6 (1926)]

COLOR AND SEX IN THE INDIAN WALKING STICK, *DIXIPPUS MOROSUS*

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In cultures of the Indian walking-stick, *Dixippus morosus*, reared in the insectary at the Bussey Institution, it was observed that in each generation there was a wide range of coloration varying from a uniform green in some individuals to a brownish black in others. Since this insect usually reproduces parthenogenetically the question as to the behavior in inheritance of the several color types appeared to be one of considerable interest.

Dobkiewicz (1912) had already shown that the color of these insects is influenced by their surroundings. He reared them in cages lined with colored paper, and found that those in green and yellow cages remained light green throughout their lives, while those in red and black cages had become quite black by the time they had reached sexual maturity. However, the occurrence in our cultures under what appeared to be a uniform environment of green and dark brown individuals, in addition to a number which were intermediate between the two extremes, suggested that there might be inherent individual differences in reactivity to a background of a given color.

To obtain further information on this question two females, one a light green and the other a dark brown, were isolated and their eggs saved. The eggs are dropped at the rate of two or three a day, and egg-laying may extend over a period of three to five months. Over one hundred eggs were obtained from each female. They were placed in wide-mouthed bottles stoppered with a cotton plug, and were stored in a damp situation in the insectary. In about five months after the first eggs were laid, hatching began.

Four cages were constructed,—two were lined with light yellowish-green cheesecloth and two with the same material of a very dark red color. After a number of eggs from both the green and the brown parent had hatched, twenty-four young from each female were selected and divided into two lots of twelve each. One lot was then placed in a green cage, the other in a red cage.

The insects at this stage were uniformly of a dull green color. They were fed each night with shoots of *Tradescantia fluminensis*, the remains of which were removed each morning to avoid obscuring the background.

No change could be observed after either the first or the second moult. After the third moult, however, those in the green cages had taken on a clearer, lighter shade of green, while those in the red cages had become perceptibly darker. After the fourth moult the difference was striking,—those in the green cages remained a clear green, while those in the red cages were dark brown. There was but little difference in the rate of change between the progenies of the two females. Those from the green parent showed, if anything, a more rapid change to brown than did those from the brown parent.

To determine whether the change to dark brown could be effected in the later stages of the life history, two individuals were transferred from each of the green cages and placed with their sisters in a red cage After passing through several moults, they became almost dark as those which had occupied the red cages from the beginning of the experiment

At the same time two brown individuals were removed from each of the red cages and were placed with their sisters in the green cages to determine whether the color change to brown is reversible. In each case the middle pairs of legs of the transferred individuals were removed to distinguish them from the others. After several moults they had taken on a somewhat lighter shade, and when mature they were of an indefinite greyish color; but the dark pigment was never completely resorbed. The middle legs, however, which were regenerated under the influence of the green background were of a peculiar bluish-green shade, their color presenting a striking contrast to that of the rest of the body.

In each of these cases no differences could be observed in the reactivity of the two progenies.

After the insects had attained sexual maturity, two other green individuals were transferred to red cages and two dark brown individuals to green cages. No color change was observed. Apparently the color present after the last moult is permanent.

The question as to the reasons for the original variations in color of individuals occupying the same environment as found in our cultures remains unanswered. The only explanation which suggests itself is the following: The insects feed during the early part of the night attaching themselves before dawn to a branch or any other suitable support, and remaining motionless in the same spot throughout the day. They show a marked tendency, in captivity at any rate, to return to the same support day after day. If the slight differences which exist in the illumination and background are sufficient to bring about the differences in color, this habit may be responsible for the observed variation.

Fryer (1913), working with a bisexual walking-stick, *Clitumnus cuniculus*, from Ceylon, in which both yellow and green forms are found, interpreted the color differences as being inherited. The color, according to his hypothesis, is due to action of a single factor pair, yellow being dominant over green His data, however, do not furnish the necessary proof for his hypothesis. The males are uniformly of the same color, and he was thus able to assign to the male used in a given mating the particular genetic constitution necessary to explain the proportions obtained in the progeny; but apparently the tests necessary to determine the correctness of his assumptions were not made.

The fact, as shown by Pantel and de Sinety (1918), that coloration in several other species of walking-sticks in addition to Dixippus, is dependent upon the environment, suggests the desirability of reexamining the behavior in *Clitumnus* from this viewpoint.

Among a total of several thousand individuals reared in our cultures, two males and one gymandromorph appeared. The sporadic occurence of males and gymandromorphs has also been reported by others. Nachtsheim (1922) has suggested that non-disjunction is responsible for their appearance. In the Orthoptera generally, the female is characterized by the presence of two X-chromosomes, the male by one. The loss of one X-chromosome by non-disjunction or in any other manner would presumably result in maleness. If the loss occurred before the first somatic division, the entire individual would be male,—if it occurred later a gynandromorph would presumably result. In the gynandromorph mentioned, the left side throughout the entire length was typically typically female, the right side was male, suggesting that the loss of an X-chromosome had occurred at the first somatic division.

One of the males was placed in a cage with several females which had just attained sexual maturity. Only females appeared in their progenies. Copulation was not observed, but the insects were seldom examined at night. Nachsheim mated one of the males which appeared in his cultures with females and observed repeated copulation but with no effect upon the sex of the progeny,—as in the progenies resulting from unmated individuals, all were females. Since, according to Pehani (1924) normal spermatozoa were produced by one of the sporadic males studied by him, it appears that the eggs have lost their capacity for fertilization.

In forms whose eggs have the ability to develop parthenogenetically and where parthenogenetic reproduction results exclusively in females, a trend in the sex ratio is automatically set up, which must end in the ultimate elimination of males unless this effect is offset by some factor or factors, such as greater viability of males or of male-producing spermatozoa, which tend to distort the sex ratio in the opposite direction. In the Orthoptera, forms having the capacity for parthenogenetic reproduction are known in which males are never found, others in which males are rare, and still others in which the sex ratio is near equality. It must be concluded that forms in the last category have developed the capacity for parthenogenesis only recently or, as already suggested, that the trend toward an excess of females, which would otherwise be an inevitable concommitant of thelyotokous parthenogenesis, is being offset by other factors tending toward an excess of males.

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MENDELIAN FACTORS PRODUCING SELECTIVE FERTILIZATION

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Reprinted from The American Naturalist, Vol. LX, March–April, 1926

Reprinted from THE AMERICAN NATURALIST, Vol. LX, March–April, 1926

MENDELIAN FACTORS PRODUCING SELECTIVE FERTILIZATION

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ONE of the fundamental assumptions of the laws of Mendelism is the theory of the free combination of all gametes of opposite sex. It forms the basis for the chance distribution of all the possible gene combinations. We have recently become acquainted with cases, however, where the attraction between two gametes of opposite sex does not depend upon this sex differentiation alone but is affected also by other Mendelian factors. Such cases are now found in very different groups throughout the plant kingdom, in Basidiomycetes, in Dicotyledons and in Monocotyledons. It can hardly be doubted that such a selection of viable gametes in some combinations is a rather general phenomenon causing deviations from expected ratios. As in cases of linkage and of elimination of gametes or zygotes having a certain constitution, it causes an excess of some combinations and a deficiency of others in the offspring. A peculiarity which it has in common with heterogamy is in causing a difference between reciprocal crosses in higher plants. There is a selection between the male gametes only.

The first carefully analyzed case was published by Correns (1917, 1918, 1921). He investigated the sex inheritance in the dioecious *Lychnis dioica* L. (*Melandrium rubrum* Garcke) and *L. alba* Mill. (*Melandrium album* Garcke). The male plants are interpreted as heterozygous (Ff) and produce two types of pollen grains; male determining (f) and female determining (F). The female plants are homozygous (FF) and form only one type of eggs (F). In the F_1 generation of a normal cross (FF × Ff) Correns does not obtain the expected sex ratio 1 FF : 1 Ff, but a deficiency of males (Ff). He was able to show that the shortage of males (Ff) was due to a slower rate of growth of the male determiners f as compared with the female determiners F.

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More recently Correns (1922) has investigated the sex inheritance of the dioecious *Rumex acetosa* L., where again the male plants are the heterozygotes (Ff) and the female plants the homozygotes (FF). As before, there appeared in the offspring of a normal cross FF × Ff an excess of the females (FF) which was due to a favoring of the female determiners F.

In both of Correns's cases, therefore, it is the pollen with the constitution F which was better able to function in the tissue of the female plant (FF) than the pollen type f.

Jones (1924) found a similar case in a cross between pop corn (SuSu) and sweet corn (susu). In the offspring of certain F_1 combinations an excess of Su plants appeared. In other crosses the expected ratio was found. A significant deviation appeared only when the pollen of the heterozygous Susu plant was used, and probably was due to the fact that in this pollen mixture the Su pollen was better able to function than the su pollen. Further, the difference in the behavior of the two pollen types was found only when females were used which carried also the Su factor, either in the homozygous or the heterozygous state. Of the three possible crosses with Susu pollen, a significant deviation appeared only in the cross Susu × Susu and the cross SuSu × Susu, but not in the cross susu × Susu, where the deviation was, according to Jones, only 1.88 times the probable error.

From these three reported cases one could draw the general conclusion that like tends to mate with like. In *Lychnis* and *Rumex* the F pollen is favored in a FF plant, in Zea the Su pollen is favored in plants of the constitution Susu and SuSu which carry also the Su factor.

The mutual attraction between like factors, however, is not a general rule. Heribert-Nilsson found an excess of red-nerved plants in the offspring of crosses made with the pollen of a heterozygous red-nerved *Oenothera Lamarckiana* of the constitution Rr on either heterozygous red-nerved plants (Rr) or homozygous white-nerved plants (rr); the homozygous red-nerved plants (RR) could not be investigated because the combination is lethal. Heribert-Nilsson was able, through a very simple method, to show (1923) that this excess was due to a slower rate of growth of the red-nerve determining pollen-tube r in the tissue of a Rr plant or a rr plant. The r tube is less likely to fertilize eggs than the R pollen-tube. In other words, unlike tends to mate with unlike.

In other Oenothera crosses, Renner (1919, 1921) explains deviations from the expected segregation also by the assumption of a selective pollen-tube growth.

Another case of a selection of gametes according to their constitution was recently published by East and Mangelsdorf (1925) as a result of the senior author's intensive studies on self-sterility in Nicotiana. They found special sterility factors acting in such a manner that pollen-tubes carrying a factor identical with a factor of the tissue in which they were growing failed normally to fertilize eggs. These sterility factors form a series of multiple allelomorphs of which three factors s_1, s_2 and s_3 have as yet been investigated in detail. For instance, normally in the offspring of a cross of a s_1s_2 plant with a s_1s_3 plant only the s_1s_3 and the s_2s_3 combinations appeared, s_1 pollen having failed to function on a s_1s_2 plant. Similarly, in the cross $s_1s_2 \times s_2s_3$ the combinations with s_2 pollen were lacking; while in the cross $s_2s_3 \times s_1s_3$ the s_3 pollen was deficient. The pollen of a homozygous plant functioned only on plants which did not contain the same factor. For instance, s_1s_1 pollen failed on s_1s_1, s_1s_2 and s_1s_3 plants, but not on s_2s_3 plants, and so forth.

These results are not always so clear as we have indicated. When bud pollinations are made, they are complicated by what has been called pseudo-fertility, where some small portion of the inhibited pollen-tubes function and produce plants in the offspring. In such cases we have not a complete absence of expected segregates, but a deficiency only, as in *Lychnis, Rumex, Oenothera* and *Zea.*

It is very interesting that an identical situation has been found by Kniep and Brunswik in Basidiomycetes. The apparent differences are from the pure genetical viewpoint superficial. They are due to the diversity in the ontogenetical development of the fungi and the higher plants.

The diploid sporophore is homologous with the diploid sporophyte in the higher plants, and forms spores following a normal reduction division. After germination these spores form haplomycelia. Through some kind of fertilization the diploid state can be restored and the diplomycelium may form again a sporophore. The cycle is then closed.

In the Phycomycetes where the alternation of generations is similar, Blakeslee and his coworkers and also Burgeff have shown that in some species the haplomycelia are homothallic or monoecious, as one would call it in diploid plants; in other species they are heterothallic. One finds then plus mycelia and minus mycelia, both produced in the reduction division in equal numbers and therefore based on the segregation of a single pair of simple alleolomorphs. Every plus mycelium can mate with every minus mycelium, whatever the origin may be.

In the Basidiomycetes Brunswik found homothallic forms, while Kniep and Brunswik investigated other species which seemed to be heterothallic. But this heterothally is not, at least not in all species, the same as in the Phycomycetes. In some forms two groups of spores and therefore of haplomycelia are formed through the reduction division, only haplomycelia of different group mating with other. But in other species four groups appear showing rather complicated sex relations. The situation is easily explained however with the assumption of two pairs of allelomorphs which are not linked and appear therefore after the reduction division in four haploid combinations in equal numbers. Only mycelia different in both loci can mate. For instance, a diplomycelium $a_1a_2b_1b_2$ forms the following four types of spores: a_1b_1, a_1b_2, a_2b_1 and a_2b_2. Of these four types only two pairs are absolutely different and can mate with each other: a_1b_1 with a_2b_2 and a_1b_2 with a_2b_1. If the original diplomycelium was obtained through the cross $a_1b_1 \times a_2b_2$, only the following two of the haplomycelia can mate with one of the parents: a_1b_1 with the parent a_2b_2 and a_2b_2 with the parent a_1b_1. All the other combinations are sterile because the haplomycelia have at least one factor in common.

In several of the species investigated the two or four groups formed by different diplomycelia are not identical as in the Phycomycetes. This difference is due, as first found by Kniep (1922), to the fact that we are dealing not with simple allelomorphs but with a series of allelomorphs. These series can be very large. Brunswik isolated 27 haplomycelia in one case (*Coprinus fimetarius* (L.) Fr.), all different from each other in two loci.

We see therefore in the *Basidiomycetes* as in *Nicotiana* multiple factors producing the effect that only gametes bearing different factors usually can mate. Again unlike tends to mate with unlike.

All the cases reported above seem to justify the following conclusion: *Mendelian factors exist which produce a selection among the gametes of a plant heterozygous for these factors; and both the mating of like with like or of unlike with unlike may be favored.*

Besides the cases reported above other data have been published which attempt to show a selective fertilization. These cases are doubtful, however, either because the number of plants investigated is too small or for other reasons. Selective fertilization should not merely be a method to explain unexpected segregations. It is a concrete theory susceptible of test in nearly every case.

While in a case of linkage between genes the deviation from the expected segregation is the same in reciprocal crosses, a selection takes place only between the pollen types. The eggs behave normally. The difference between the elimination of gametes and selective fertilization lies in the fact that the first is fixed for a given plant, the latter appears only in particular combinations. The gametes here are always potentially functional and fail in certain combinations only. *The selection is due to an interaction between the genotype of the female diploid plant and the male gametophyte in higher plants or between the two gametes in the Basidiomycetes.*

This interaction has been proved in the *Basidiomycetes* and in *Nicotiana,* where gametes of the same constitution do not mate; and in the cross between pop corn and sweet corn, where the stimulation of the Su pollen is found only when the Su factor is also present in the female plant. It was not susceptible of proof in *Lychnis* and *Rumex* because the female plants have always the same constitution, and in the red-nerved *Oenothera* in which the homozygous red-nerved plants are lethal and the heterozygous red-nerved and the white-nerved plants alone can be investigated. In the latter case we can assume by analogy with the experiments on other plants that it is the selective action of the r factor present in the male and the female plants of all crosses that produce the change in the ratio.

Further, in higher plants one may assume that *it makes a difference whether the factor for selective fertilization is present in the female in the homozygous or the heterozygous condition.* In the first case one would expect a more stringent selection. In species where a deficiency of some segregates is the result of the selection of gametes, in the plant homozygous for the selective factor, the deviation from the expected segregation should be higher than in a heterozygous plant.

In maize, using the data of Jones, the deficiency of the homozygous recessives in the cross Susu × Susu is 8.8 per cent. (probable error ± 0.45 per cent.), and in the cross SuSu × Susu the deficiency is 21.6 per cent. (probable error ± 0.95 per cent.). The difference between the two deviations is 12.8 per cent. ± 1.02 per cent.

In the red-nerved Oenothera we find in the data of Heribert-Nilsson a deficiency of the homozygous recessive white-nerved plants of 12.7 per cent. (probable error ± 1.69 per cent.) in the cross Rr × Rr and a deficiency of 23.2 per cent. (probable error ± 1.81 per cent.) in the cross rr × Rr. The difference is 10.5 per cent. ± 2.88 per cent.

In both cases the deviation from the expectation is higher in the homozygous female than in the heterozygous. The difference between the two deviations is more than three times the probable error and therefore significant.

Finally, two general features of the behavior of the factors for selective fertilization should be mentioned because they are important from the viewpoint of genetical theory.

The degree of the selection varies greatly phenotypically as well as genotypically. The individual sets of observations of Correns, Jones and of Heribert-Nilsson show a variability which is higher than if due to chance alone and may in part be phenotypical, in part be caused by modifying genes. In self-sterile plants pseudo-fertility is known as a general phenomenon (Stout). East and Park (1917) found in Nicotiana an end-season-pseudo-fertility. In some species of Coprinus Brunswik found cases of a very clear pseudo-fertility where haplomycelia which should not mate fertilize each other to some extent.

The factors for selective fertilization themselves show in some cases a relatively high mutability. Such a mutability seems to be an explanation for the appearance of a large series of multiple allelomorphs, as found in the Basidiomycetes in several species. Kniep (1923) was able to detect in the Basidiomycete *Aleurodiscus polygonius,* which has two series of allelomorphs, in relatively small numbers of plants mutations in all four allelomorphs of an original diplomycelium. The figures are however too small to calculate the mutation percentage.

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Connecticut Agricultural Experiment Station, New Haven, Conn.

Reprinted from GENETICS **11**: 423-455, September, 1926

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Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879.

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Received March 1, 1925

TABLE OF CONTENTS

	PAGE
Distorted ratios in defective seeds	423
Interpretation of defective ratios	428
Distorted ratios in sugary endosperm	433
Linkage relations between *Su* and de_1	437
Relative effectiveness of *Ga* and *ga* gametes	437
Predicting the size of deviations	442
Gametophyte factors and mixed pollinations	443
Distorted ratios in waxy endosperm	445
Analysis of KEMPTON's data	446
Peculiar ratios in color segregation	448
How gametophyte factors operate	449
Carbohydrate metabolism and pollen tube growth	451
DISCUSSION	451
SUMMARY	453
LITERATURE CITED	454

DISTORTED RATIOS IN DEFECTIVE SEEDS

In 1918 a number of plants of a large-seeded, yellow, variety of flint corn, known as Gold Nugget, were self-pollinated. Nineteen selfed ears were secured and among these were three which segregated for a type of endosperm abnormality to which has been applied (JONES 1920) the term "defective seeds". Defective seeds may be described as lethal or semi-lethal characters in which the endosperm and embryo are greatly reduced in size or almost completely lacking. These characters appear very frequently when maize is inbred and it has been shown (MANGELSDORF 1923, 1926) that there are many genetically distinct types of this abnormality.

The defective which appeared in the Gold Nugget variety and to which the factor symbol de_1 has been given, is a semi-lethal type. The recessive seeds are characterized by a smaller size and aborted, shrivelled appearance. Their development is about half that of normal seeds on the same ears and they can, in most cases, be readily and accurately separated from the latter.

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[1] Also contribution from the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

In 1920 the strain carrying the de_1 factor was crossed with a defective from another variety. In the F_2 endosperm generation of this cross, three ears segregating for defective seeds of the de_1 type were obtained. These ears bore 567 normal and 194 defective seeds, almost a perfect 3:1 ratio. The natural conclusion was that this character is inherited as a simple Mendelian recessive.

In 1922 a de_1 strain of slightly different pedigree from the one used in 1920, but closely related to the latter, served as a pollen parent in crosses with five different unrelated stocks. In the F_2 generation of these crosses fourteen progenies segregating for the parental de_1 type were recovered.

TABLE 1

Progenies segregating for de_1 in the F_2 endosperm generation of crosses made in 1922.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
22	160	49	−3	.7	23.4
24	154	63	9	2.1	29.0
25	183	90	22	4.6	33.0
42	184	55	−5	1.2	23.0
126	428	126	−15	2.2	22.7
128	265	90	1	.2	25.4
129	369	180	43	6.3	32.8
173	200	93	20	4.0	31.7
388	498	197	23	3.0	28.3
389	424	155	6	.9	26.8
391	127	61	14	3.5	32.4
392	328	82	−21	3.6	20.0
393	284	97	2	.3	25.5
1520	346	175	45	6.8	33.6
Total	3950	1513	147	6.8	27.7

When the counts of normal and defective seeds on these ears were combined (table 1) it was found that the defectives occurred in excess of the theoretical expectation. The average percentage of defectives in this group was 27.7. This represents a deviation from a 3:1 ratio of 6.8 times the probable error and is undoubtedly significant. A deviation of this size should occur, by chance alone, only once in several hundred thousand trials. The excess of recessives is especially noteworthy because defectives of other types frequently show small deficiencies (MANGELSDORF 1926).

The reason for the difference between the percentage of recessives in the 1920 and 1922 crosses apparently lies in the different strains of de_1 which were used as parents. Table 2 shows three successive generations of segregating ears of the original Gold Nugget strain. The proportion of normal to defective seeds in this strain had always approximated a 3:1 ratio and the average percentage of recessives for the entire group is 25.5. It is noted, however, that the two of the ten ears showed an excess of defectives of three or more times the probable error. It so happens that one of these excess ears, 105-9-8-2, was used as the pollen parent in the crosses made in 1922, before the significant deviation from a normal ratio had been noted. The 1920 cross, however, which gave normal segregation in F_2 was made with plants grown from ear 105-9-7 which itself segregated normally. Apparently the excess of recessives in the second generation of the 1922 crosses traces back to some condition which caused an excess in the parental ear.

TABLE 2

Segregating progenies of three successive generations of inbreeding of a "defective" strain of Gold Nugget.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
105–9	250	73	−8	1.5	22.6
105–9–3	117	49	8	2.2	29.5
105–9–4	159	46	−5	1.2	22.4
105–9–5	146	44	−3	.7	23.2
105–9–6	185	49	−9	2.0	20.9
105–9–7	243	75	−5	1.0	23.6
105–9–8	180	78	14	3.0	30.2
105–9–9	213	66	−4	.8	23.7
105–9–8–1	202	61	−5	1.1	23.2
105–9–8–2	134	84	30	7.0	38.5
Total and Average	1829	625	11	.8	25.5

The average percentage of defective seeds, 27.7 percent, in the fourteen ears shown in table 1 represents a significant excess but is not as great a deviation as occurred in the parental ear. When the ears in this group are considered separately, however, it is noted that seven of them show no significant departures from a 3 : 1 ratio; one shows a deficiency and the remaining six an excess greater than three times the probable error. Deviations of three times the error are expected, by chance alone, fairly frequently but in this case half of the ears show marked departures. Clearly the group is not a homogeneous one.

When the six ears which show significant plus deviations are combined, the average for the group is 31.9 percent recessives as compared to 25.1 percent for the non-deviating ears and 20.0 percent for the single ear with a significant minus deviation. In other words this lot of fourteen ears can be divided, on the basis of the deviations, into three distinct groups with *high*, *normal* and *low* defective ratios. A deviation of three times the probable error has been arbitrarily chosen as the dividing point.

If the *high* and *low* defective ears are merely the result of chance deviations, then progenies grown from such ears should give approximately normal ratios in F_3. If, however, these deviations have a genetic basis, distorted ratios might again be expected in F_3.

TABLE 3

Segregating F_3 progenies from "high defective" F_2 ear No. 391.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
813	174	85	20	4.3	32.8
822	227	99	18	3.4	30.4
Total	401	184	38	5.4	31.5
Progenies with significant deficiency					
810	108	15	−16	4.9	12.2
811	319	63	−33	5.8	16.5
815	235	56	−17	3.4	19.2
Total	662	134	−65	7.9	16.8
Progenies with no significant deviation					
817	275	83	−7	1.3	23.2
819	130	55	9	2.3	29.7
820	273	89	−2	.4	24.6
Total	678	227	1	.1	25.1
Grand Total	1741	545	−27	1.9	23.8

Third generation progenies of two of the *high* defective ears, 391 and 1520, have been grown and the results are presented in tables 3 and 4.

Twelve progenies were obtained from ear 391 (32.4 percent recessives in F_2), of which eight were segregating for defective seeds. Although the average percentage of recessives for this group is approximately normal, 23.8 percent, it is noted that five of the eight ears deviate from a normal ratio by amounts greater than three times the probable error. Two ears are *high* with an average of 31.5 percent defectives, three are *low* with an average of 16.8 percent recessives and the remaining three are *normal* with an average of 25.1 percent.

In connection with a possible explanation of the distorted ratios in waxy endosperm, presented later, particular attention is called to the difference between the percentage of defectives in the parental F_2 ear (32.4), and the average percentage of defectives in its progenies (23.8). The average of the F_3 progenies approached closely the normal expectation and it is only when the ears are considered separately that irregularities are noted.

Seventeen F_3 progenies were obtained from ear 1520 (33.6 percent recessives in F_2), of which ten were segregating. Four of the segregating ears were *high* with an average of 32.8 percent defectives, one *low*, with 16.8 percent, and five were *normal* with 25.3 percent defectives.

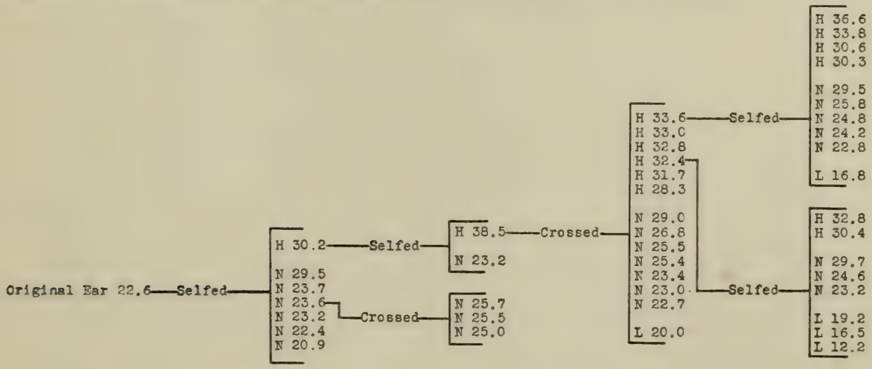

FIGURE 1. Pedigree record of the *de*$_1$ stock showing the inheritance of deviations from normal ratios. The letters H and L represent ears with plus or minus deviations greater than three times the probable error. N represents ears in which the deviation is less than three times the error.

Combining the 18 segregating progenies from these two high defective ears we find that six are *high* with an average of 32.4 percent recessives, four are *low* with 16.8 percent recessives and eight are *normal* with 25.2 percent recessives. The average for the entire F_3 group is 26.4 percent as compared to 33.3 percent for the two parental F_2 ears, a difference of practically seven percent between F_2 and F_3.

Obviously the marked departures from normal ratios in ten of the eighteen F_3 ears cannot be attributed to chance alone. Nor can the results be explained by faulty classification since errors of this sort are usually consistently in one direction. That the marked deviations have a genetic basis is indicated by the fact that they appear in four separate generations as is shown in figure 1.

Apparently the original stock contained a factor or factors which in some way disturbed the normal segregation. The action of this factor first became apparent in the second generation of inbreeding. It has since been transmitted to later generations both in selfed lines of the original stock and in crosses.

TABLE 4

Segregating F_3 progenies from "high defective" F_2 ear No. 1520.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
1788	224	99	18	3.5	30.6
1790	156	90	28	6.1	36.6
1792	365	186	48	7.1	33.8
1806	205	89	15	3.0	30.3
Total	950	464	109	10.0	32.8
Progenies with significant deficiency					
1794	188	38	−18	4.1	16.8
Progenies with no significant deviation					
1795	219	70	−2	.4	24.2
1796	167	58	2	.5	25.8
1797	85	28	0	..	24.8
1802	170	71	11	2.4	29.5
1803	227	67	−7	1.4	22.8
Total	868	294	4	.4	25.3
Grand Total	2006	796	96	6.2	28.4

INTERPRETATION OF DEFECTIVE RATIOS

Whenever marked deviations from expected ratios are encountered the possibility of lethal factors at once suggests itself. In maize, however, where a large number of seeds are arranged in a very regular fashion on a single inflorescence, lethal factors, or any sort of differential mortality of the zygotes are readily noted by the occurrence of aborted kernels scattered over the ear. The defective seed in question is, in fact, itself a semi-lethal character.

Lethal factors, moreover, should cause an excess of recessives only when they are linked with the dominant allelomorph of the recessive in question. The marked plus deviations in six of the F_2 ears shown in table 1 could be explained on the basis of lethal factors, only by assuming that each of the five separate seed parents involved in these crosses was heterozygous for a lethal factor linked with the dominant allelomorph of the de_1 gene. The possibility of lethal factors can, therefore, be safely ruled out.

The next hypothesis to be considered is that of differential pollen tube growth. The fact that defectives of other types had frequently given slight deficiencies suggested that these characters, which have such a profound deleterious influence on the sporophyte, might also have some effect in the gametophyte generation, particularly on the rate of pollen tube growth. To test this hypothesis a large number of ears segregating for various types of defectives were arbitrarily divided into upper and lower halves and the proportion of defectives in each half determined separately. If the two types of pollen tubes differ in their rate of growth it might be expected that the greater distance which the tubes were required to travel in reaching the ovules at the base of the ear, would react in favor of the faster growing tubes; that the longer the race the more certain the success of the faster individuals.

Table 5

Segregation in upper and lower halves of nine "high defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	Upper	Lower	
25	75	35	108	55	31.8	33.7	1.9
129	179	83	190	97	31.7	33.8	2.1
173	101	49	99	44	32.7	30.8	−1.9
388	246	91	252	106	27.0	29.6	2.6
391	77	32	50	29	29.4	36.7	7.3
1788	108	45	116	54	29.4	31.8	2.4
1790	77	43	79	47	35.8	37.3	1.5
1792	189	92	176	94	32.7	34.8	2.1
1806	106	40	99	49	27.4	33.1	5.7
Total	1158	510	1169	575	30.6	33.0	2.4

The results of these counts indicated that, with most of the defective types, there was no appreciable difference between the upper and lower halves of the ear in proportion of defectives, and that the defective seed factors do not *per se* reduce the rate of pollen tube growth.

It happened, however, that several of the *high* and *normal* ears of the de_1 stock were included in this particular study, without realizing at the time that some of these ears were exceptional. The counts on these ears and several additional ones which have since been examined are shown in tables 5 and 6.

In the *high* ears the average percentage of defectives in the upper halves was 30.6 as compared to 33.0 in the lower halves of the same ears. This difference, though not great, appears to be significant, the odds against its chance occurrence being about 70 to 1 (calculated by Student's formula). The fact that the difference is consistent, all the ears in table 5 with one exception showing a greater proportion of recessives in the lower halves, is regarded as particularly important.

The *normal* ears on the other hand (table 6) show differences in favor of the upper halves as frequently as the lower and the averages of the two halves are very closely alike, 24.6 and 25.5 percent. These results, so far as they go, point to differential rate of pollen tube growth in the *high* ears and equal rate of pollen tube growth in the *normal* ears.

TABLE 6

Segregation in upper and lower halves of seven "normal defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	UPPER	LOWER	
22	84	27	76	22	24.3	22.4	−1.9
24	75	32	79	31	29.9	28.2	−1.7
42	91	27	93	28	22.9	23.1	.2
126	211	54	217	72	20.4	24.9	4.5
128	142	52	123	38	26.8	23.6	−3.2
389	235	75	189	80	24.2	29.7	5.5
393	160	59	124	38	26.9	23.5	−3.4
Total	998	326	901	309	24.6	25.5	.9

Apparently the differential rate of growth is not due to the action of the de_1 factor itself. If pollen tubes carrying this gene were regularly faster or slower in rate of growth, the deviations should regularly occur in the same direction in *all* of the ears. This is not the case. Some ears have marked minus deviations, some marked plus deviations and the remainder segregate normally.

These peculiar results may, however, be explained by assuming that the chromosome pair which carries the De_1 and de_1 factors also bears a gene which influences the rate of pollen tube growth. Since this hypothetical factor has its expression in the gametophyte generation we may give it the factor symbol *Ga*. This factor speeds up the rate of growth in the pollen tubes which carry it and, in the original stock, was linked with de_1, thus enabling a larger proportion of de_1 gametes to accomplish fertilization than would occur by chance alone.

On this assumption the *high* defective plant which served as pollen parent for the 1922 crosses was of the genetic composition $Gade_1$, $gaDe_1$. Such a plant would produce four kinds of gametes, *Gade*, *gaDe*, *gade*, and *GaDe*. The seed parents of these crosses contributed only one kind of gamete *gaDe*. Thus four distinct classes of F_1 plants would be produced as follows:

1. *gaDe GaDe*
2. *gaDe gaDe*
3. *gaDe Gade*
4. *gaDe gade*

These four types would not be expected to occur in equal numbers because their proportion depends upon the amount of crossing over between *Ga* and *de* in the original stock.

Classes 1 and 2 are homozygous for the dominant De_1 factor and need not be considered further since they do not segregate for defective seeds. Plants in class 4 give segregating ears but the ratios should be normal because the plants are homozygous for *ga* and no differential pollen tube growth can occur. Plants of class 3, however, should, when selfed, give only *high* defective ears because both factors are in the heterozygous condition and the *Ga* factor which speeds up the rate of pollen tube growth is linked with the de_1 factor which causes defective seeds, thus enabling a larger proportion of the de_1 gametes to accomplish fertilization than would occur by chance alone.

A glance at table 1 shows that this is exactly the situation in the fourteen F_2 ears. Seven *normal* and six *high* ears resulted from these crosses. In addition, however, there is one *low* ear. *Low* ears are not expected from these particular crosses and it is not certain whether ear 392 with 20.0 percent recessives is an exception to theory or merely a chance variation from a *normal* ratio.

It is obvious that in dividing the ears into *high*, *low* and *normal* on the basis of the size of their deviations, some ears which have plus or minus deviations due to chance alone and not to genetic factors, will be included in the two extreme groups. Also some ears which are genetically *high* or *low* will be classed as *normal* because the number of seeds counted is not sufficient to establish the significance of the deviations.

What should happen in F_3 when the F_2 seeds from a *high* defective ear are grown? The *high* ear, we assume, was borne on a plant of the composition *Gade gaDe*. Such a plant would form four kinds of gametes in the pollen as well as in the ovules and self-pollination should give ten classes of seeds. The genetic constitution of these ten classes and their theoretical behavior when grown and self-pollinated is shown below:

GENOTYPE	BEHAVIOR
1. *GaDe GaDe*	Does not segregate
2. *GaDe gaDe*	Does not segregate
3. *gaDe gaDe*	Does not segregate
4. *GaDe Gade*	Segregates; 3 : 1 ratio
5. *gaDe gade*	Segregates; 3 : 1 ratio
6. *Gade gaDe*	Segregates; *high* defective ratio
7. *GaDe gade*	Segregates; *low* defective ratio.
8. *Gade Gade*	Defective seed
9. *Gade gade*	Defective seed
10. *gade gade*	Defective seed

Genotypes 1, 2, and 3 are homozygous for De_1 and do not segregate. Genotypes 8, 9, 10 are defective seeds and will not grow. Genotypes 4 and 5 segregate but both give normal ratios because the plants are homozygous for *Ga* or *ga* and differential pollen tube growth cannot occur. Plants in genotype 6 are heterozygous for both factors and, since *Ga* and de_1 are linked, should give *high* defective ears. Genotype 7 represents a new combination in which the linkage relations have been reversed. Here the *Ga* factor is linked with the dominant allelomorph and instead of an excess of defectives we should expect a deficiency.

Briefly summarized, then, the seeds from a *high* F_2 ear should, when grown and selfed, give *high*, *low*, and *normal* F_3 progenies. The 18 segregating ears in tables 3 and 4 meet these requirements very satisfactorily. Six are *high*, eight are *normal* and four are *low*. The ratio in which these three types of segregating progenies appear depends not only on the amount of crossing over between *Ga* and de_1 but on the ratio in which the *Ga* and *ga* gametes are presented at fertilization.

It should be emphasized that the validity of this interpretation does not necessarily depend upon the assumption of differential pollen tube growth. Any factor which affects the proportion in which the gametes are presented at fertilization would act in the same manner although its effect might be produced in a number of ways, as for example, by causing differences in the duration of viability or resistance to unfavorable moisture and temperature extremes.

The important fact is that this stock apparently *does* carry a factor which disturbs normal segregation and that this factor is transmitted from generation to generation as is any other Mendelian character. *How* the factor produces its disturbances is another question and one which seems to be partially answered by the data which point to differential pollen tube growth.

DISTORTED RATIOS IN SUGARY ENDOSPERM

The hypothesis of an accessory factor causing differential rate of pollen tube growth has been adopted because the situation is almost identical to that encountered when sugary endosperm is crossed with certain small seeded corneous varieties of the *Zea maize everta* type, known as Squirrel Tooth or Rice Pop.

Although sugary is usually inherited as a simple recessive and gives ratios closely approximating expectation, the Rice Pop × sugary crosses are exceptional in that they always produce a marked deficiency of sugary seeds in the F_2 endosperm generation. This deficiency was first noted by CORRENS (1902) who reported 16 percent sugary seeds in the second generation. EAST and HAYES (1911) also noted the same peculiarity and suggested that the disturbance might be due to faulty classification or to unknown characters contributed by the parents. JONES (1924) and EMERSON (1925) have both made detailed studies of this cross and both have come to the conclusion that the aberrant ratios may be attributed to differential pollen tube growth. EMERSON, however, suggested that the differential rate of growth is not due to the *Su* gene itself but to an accessory factor on the same chromosome. The details of EMERSON'S interpretation of this cross have not yet been published and it is not known whether his explanation agrees with the one given here.

The results of the genetic studies of the sugary × pop cross at the CONNECTICUT STATION are briefly summarized in the following paragraphs.

An inbred strain of pointed pop corn was crossed with a first generation hybrid of two inbred strains of sweet corn. One of the sweet strains was a small yellow-seeded variety known as Golden Bantam, the other a large white-seeded variety known as Evergreen. This material was used because it had shown a high degree of selective action in pollen mixtures where pollen from two different types of plants, acting in competition, fertilized more of the ovules of its own type than of the diverse types as previously reported by JONES. (1920, 1922).

In a total of 3681 seeds from eight self-pollinated F_1 plants of this cross shown in table 7, there are only 16.2 percent of sugary seeds. This represents a departure from the normal ratio of 18.3 times the probable error. The percentages of recessives in the individual ears range from 10.5 to 21.4 and the deficiency is greater than three times the probable error in all but one of the ears. In this ear the deviation is 2.6 times the probable error.

F_1 plants of similar cross, though of somewhat different ancestry were backcrossed both ways with the recessive parent. In the backcross of the heterozygote with pollen from the recessive parent (*Susu* × *susu*), 1374 starchy and 1397 sugary seeds were produced. In the reciprocal backcross (*susu* × *Susu*) 885 starchy and 939 sugary seeds occurred. The deviation from a 1 : 1 ratio is not significant in either case. In this respect the results differ from observations with waxy corn which are discussed later. In all this material the segregation of smooth, corneous seeds and wrinkled, glassy seeds was very clear-cut. Occasionally a recessive seed was found that was not deeply wrinkled and at first glance might be classed as a dominant. However, the opportunity for faulty classification was just as great in the F_1 plants backcrossed with the recessive as in the F_1 plants self-pollinated. In the one case a marked deviation was found; in the other, not.

Table 7

F_2 progenies of a cross between sugary endosperm and Squirrel Tooth Pop.

EAR NO.	STARCHY	SUGARY	DEVIATION	DEVIATION / P.E.	PERCENT SUGARY
(*Su*×*su*) —1	366	43	−59	10.0	10.5
2	346	94	−16	2.6	21.4
3	376	65	−45	7.3	14.7
(*su*×*Su*) —1	322	56	−39	6.9	14.8
2	378	77	−37	5.9	16.9
3	421	98	−32	4.8	18.9
4	391	47	−63	10.3	10.7
5	485	116	−34	4.8	19.3
Total	3085	596	−324	18.3	16.2

The next step was to backcross the F_1 plants with the dominant parent (*Susu* × *SuSu* and *SuSu* × *Susu*). Since the result of fertilization by a recessive or dominant carrying gamete would be obscured, because all the seeds would have the dominant endosperm condition, it was necessary to grow the plants and determine which were segregating and which were not. This was done by planting the backcrosses, made both ways, and allowing all of the plants to interpollinate naturally. Any plants that resulted from *Su* × *Su* fertilization would show no recessives, while most of the plants of the composition *Su* × *su* would be expected to show some recessive seeds. With no selective action one-half of the plants would be segregating so that one-fourth of the pollen in the field would be carrying the recessive factor. Segregating plants would be expected to have half of their ovules with the recessive factor so that segregating ears should have, on the average, one-eighth of their seeds recessive. Selective pollination working against the recessive gametes would reduce this proportion. Only one ear was taken from each plant and most of these contained several hundred kernels. All ears which had any sugary seeds were classed as segregating. The number of sugary seeds varied on different ears but the number of plants which had no recessive seeds and for that reason would be wrongly classified was probably less than seven percent and was not likely to be enough to disturb the results seriously in view of the wide departure from the normal 1 : 1 ratio obtained. The backcross of pollen from the homozygous dominant parent on the heterozygous F_1 plants (*Susu* × *SuSu*) gave 207 segregating and 213 non-segregating individuals. These numbers differ from a 1 : 1 ratio less than the probable error. But the pollen from the heterozygous F_1 plants backcrossed on the homozygous dominant parent (*SuSu* × *Susu*) gave 88 segregating and 353 non-segregating individuals, a deviation from a 1 : 1 ratio of 18.7 times the probable error.

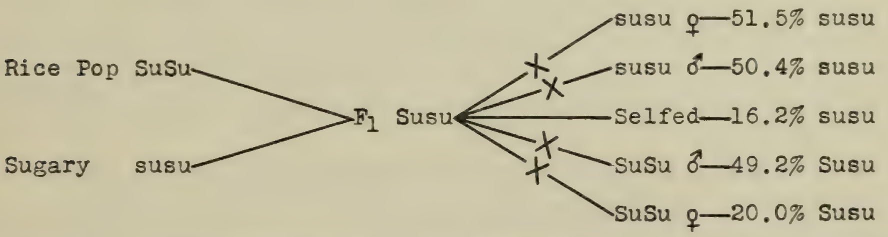

FIGURE 2. Diagram showing the behavior of the Rice Pop×Sugary cross when selfed and backcrossed reciprocally with both parents.

To sum up, the F_1 plants self-fertilized showed a selective action such that more of the gametes carrying the dominant factor united than would be the case in random mating. The heterozygous F_1 plants backcrossed with the recessive parent showed no selective action either way the pollinations were made. Backcrossed with the dominant parent there was no selective action when the pollen was alike but the pollen from the heterozygous F_1 plants showed a markedly greater pollinating ability on the part of the gametes carrying the dominant factor. These results are shown graphically in figure 2.

This series of facts shows that the selective action is not due to differences in the functioning of the pollen alone, although the differential action is exhibited only when the pollen is diverse, otherwise unequal numbers would be obtained in the backcross on the recessive parent as well as on the dominant.

As a result of these experiments JONES (1924) concluded that: "There is apparently an interaction between the pollen tube and the tissues in which it grows, such that pollen carrying the dominant factor is better able to accomplish fertilization than the pollen carrying its recessive allelomorph, only in a sporophyte which also has the dominant factor either in the haploid or diploid state."

At that time it was thought that the *Su* factor itself was responsible for the speeding up of the pollen tubes in certain combinations. It now seems more probable that the results are due to linkage of the *Su* factor with a gametophyte factor (*Ga*). If the linkage relations between *Su* and *Ga* were reversed the results would be entirely different but the above statement as applied to the *Ga* factor would still hold.

The factor symbol for the gametophyte factor has been written in capital letters as though it were a true dominant. It may be questioned whether this is legitimate since the *Ga* factor has its expression in the gametophyte where it necessarily occurs in a haploid condition and is not required to compete with its allelomorph. The *Ga* pollen tubes are given an advantage, however, only when the sporophytic tissue in which they grow also has this factor, and the fact that in the sporophyte it is apparently as effective when heterozygous as when homozygous indicates that it is a dominant factor.

On the basis of a gametophyte factor linked with the *Su* factor, the combinations here discussed may be given the following factorial representation:

TYPE OR COMBINATION	COMPOSITION OF PLANT	MALE GAMETES			
Rice Pop parent	*SuSuGaGa*	*SuGa*			
Sugary parent	*susugaga*	*suga*			
F_1 selfed	*SusuGaga*	*SuGa*	*Suga*	*suGa*	*suga*
$F_1\times$sugary parent	*SusuGaga*	*suga*			
Sugary parent $\times F_1$	*susugaga*	*SuGa*	*Suga*	*suGa*	*suga*
$F_1\times$starchy parent	*SusuGaga*	*SuGa*			
Starchy parent$\times F_1$	*SuSuGaGa*	*SuGa*	*Suga*	*suGa*	*suga*

With this factorial composition in mind the results reported in the preceding paragraphs are readily understood on the assumption that the *Ga* factor is effective in speeding up pollen tube growth only in a sporophyte which has the *Ga* factor in the homozygous or heterozygous state.

The F_1 selfed shows a selective action because *Ga* and *ga* pollen tubes compete with each other in sporophytic tissue which has the *Ga* factor in the heterozygous condition. The *Ga* tubes are given an advantage under these conditions and since *Ga* and *Su* are linked, more *Su* than *su* gametes, reach the micropyle and accomplish fertilization. F_1 plants pollinated by the sugary parent show no selective action because only one type of male gamete is involved. The backcross of the sugary parent by the F_1 involves four types of male gametes but no selective action is shown because the sporophyte in which the pollen tubes must grow lacks the dominant *Ga* factor. F_1 backcrossed by the starchy parent shows no selective action because only one kind of male gamete is present. But the backcross of the starchy parent by the F_1 again shows a very marked selective effect because *Ga* and *ga* gametes are competing in sporophytic tissue which has the dominant *Ga* factor in the homozygous state.

The F_2 progenies which show such a marked deficiency of sugary seeds, should, when selfed, produce the same three types of segregating progenies as did the *high* defective ears when selfed. Here of course the proportion of the three types would be reversed and (1) the majority should be deficient in sugary seeds, (2) some should segregate normally, and (3) a few should give an excess. EMERSON has grown such progenies and has obtained exactly these results, his *low* ears giving 15 percent recessives, his *high* ears 35 percent. The writers have also grown a few F_3 progenies from this cross and have obtained the expected types, although in the case of the *high* sugary ears the number of seeds counted was not great enough to prove that the deviations were statistically significant.

LINKAGE BETWEEN *Su* AND de_1

The question at once arises whether the deviations found in the de_1 stock are caused by the same accessory factor, always present in Rice Pop, which causes such marked disturbances in the starchy: sugary ratio. To determine this point crosses of *high* defective × pop, *low* defective × pop, *high* defective × sugary, and *low* defective × sugary have been made and the F_2 endosperm generation of these crosses will be available in another season. In the meantime, indirect evidence that the accessory factor in both stocks is probably the same is available in a cross of *normal* defective × sugary, which had been made for another purpose. Four F_2 progenies of this cross are shown in table 8.

It is noted that the two parental classes are in excess, while the two new combinations show a deficiency. When parental classes are combined and compared to the new combinations in the form of a 10 : 6 ratio, the deviation is found to be 4.9 times the probable error. Such a deviation should occur by chance alone only once in 1052 trials but can readily be explained by assuming linkage (repulsion phase) between *su* and de_1. The percentage of crossing over as determined from the normal seeds is 38.5; determined from the defective class it is 39 percent.

RELATIVE EFFECTIVENESS OF *Ga* AND *ga* GAMETES

Knowing that the de_1 and *su* factors are linked and that both are affected to a marked degree by an accessory factor located on the same chromosome, it is almost certain that the same accessory factor is acting in both stocks. On such an assumption it is possible to estimate approximately the position on the chromosome of the *Ga* factor in relation to the *su* and de_1 genes and at the same time to determine roughly the ratio of *Ga* to *ga* gametes which accomplish fertilization.

The presence of gametophyte factors which disturb the equality in which two kinds of gametes are presented at fertilization, brings up entirely new problems in Mendelian segregation. The amount of crossing over between a gametophyte factor and a sporophyte factor may range from 0 to 50 percent. At the same time the ratio in which the *Ga* and *ga* gametes occur at fertilization may range from 1 : 1 to 1 : 0.

TABLE 8

Segregation in F_2 of a cross between sugary endosperm and defective seed.

EAR NO.	NORMAL SEEDS		DEFECTIVE SEEDS	
	Starchy	Sugary	Starchy	Sugary
947	71	36	42	10
951	213	89	75	27
953	219	81	85	18
956	98	32	45	9
Total	601	238	247	64
Ex. 9 : 3 : 3 : 1	647	216	216	72
Deviation	−46	22	31	−8

Grouping into a 10 : 6 ratio

Found	665	485
Ex. 10 : 6	*719*	*431*

Deviation 54±11.1
Dev./P. E. 4.9

The amount of crossing over and the relative effectiveness of the accessory factor are, then, the two variables which determine the size of the deviations from normal ratios. Table 9 shows the theoretical percentage of recessives which should occur with different combinations of these two variables, assuming that a gametophyte factor *G* which increases the chances of the gametes, in which it occurs, to accomplish fertilization, is linked with the dominant allelomorph *S* of a recessive sporophyte factor. If the linkage were reversed (*Gs gS*), the deviations would be of the same magnitude but would occur in the opposite direction. The size of the deviations produced by different combinations of the two variables is also illustrated in figure 3.

The average percentage of defective seeds in the *high* ears of tables 3 and 4 is 32.4, a deviation of 7.4 percent from normal. The *low* ears from the same families average 16.8 percent recessives, a deviation from normal of 8.2 percent. The average deviation for both groups is 7.8 percent. In the *low* sugary ears of table 7 the average deviation from a normal ratio is 8.8 percent.

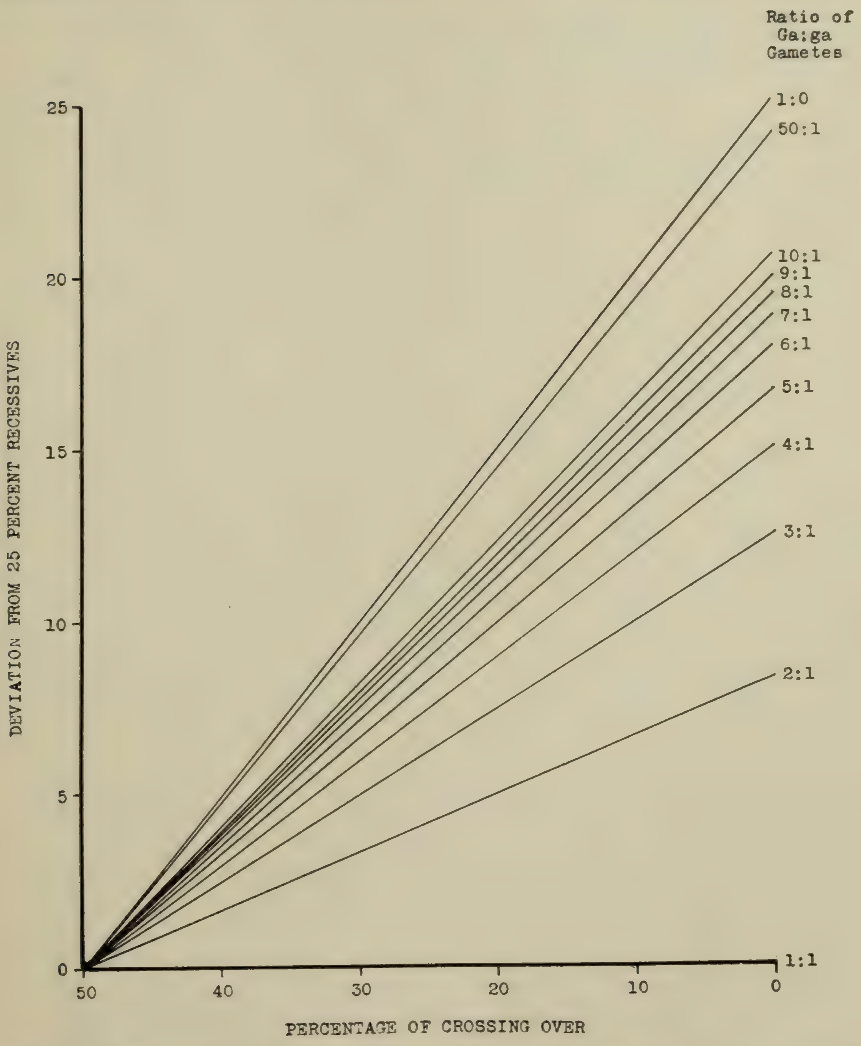

FIGURE 3. Diagrams showing the theoretical deviations from 25 percent recessives resulting from various combinations of crossing over and relative effectiveness of two kinds of gametes in accomplishing fertilization.

A deviation of 7.8 percent in the defective seed ratios and 8.8 percent in the sugary ratios might be caused by any number of combinations of crossing over and differential effectiveness of the *Ga* and *ga* gametes. This is illustrated by the curves in figure 3. Thus if the value of one of these influences were known it would be a fairly simple matter to determine the value of the other. Unfortunately this is not the case.

It is known, however, that the crossing over between *Su* and de_1 is approximately 39 percent. An accessory factor could affect the two almost equally, only if it were approximately the same distance from both. This means that its locus must lie between the *Su* and de_1 loci.

TABLE 9

Percentages of recessives (ss) expected when a factor G which stimulates the rate of pollen tube growth is linked with the dominant allelomorph S of a recessive character s.

RATIO IN WHICH GAMETES ARE PRESENTED	PERCENTAGE OF CROSSING OVER BETWEEN *G* AND *S*									
	0	5	10	15	20	25	30	35	40	45
1 : 1	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2 : 1	16.65	17.50	18.33	19.17	20.00	20.83	21.67	22.50	23.33	24.17
3 : 1	12.50	13.75	15.00	16.25	17.50	18.75	20.00	21.25	22.50	23.75
4 : 1	10.00	11.50	13.00	14.50	16.00	17.50	19.00	20.50	22.00	23.50
5 : 1	8.33	10.00	11.67	13.33	15.00	16.67	18.33	20.00	21.67	23.33
6 : 1	7.14	8.93	10.72	12.50	14.29	16.07	17.86	19.64	21.43	23.22
7 : 1	6.25	8.13	10.00	11.88	13.75	15.63	17.50	19.37	21.25	23.12
8 : 1	5.56	7.50	9.45	11.39	13.33	15.28	17.22	19.17	21.11	23.06
9 : 1	5.00	7.00	9.00	11.00	13.00	15.00	17.00	19.00	21.00	23.00
10 : 1	4.55	6.59	8.64	10.68	12.73	14.77	16.82	18.86	20.90	22.95
20 : 1	2.38	4.64	6.91	9.17	11.43	13.69	15.95	18.21	20.48	22.74
30 : 1	1.61	3.95	6.29	8.63	10.97	13.31	15.64	17.98	20.32	22.66
40 : 1	1.22	3.60	5.98	8.35	10.73	13.11	15.49	17.87	20.24	22.62
50 : 1	.98	3.38	5.78	8.19	10.59	12.99	15.39	17.79	20.20	22.60
1 : 0	.00	2.50	5.00	7.50	10.00	12.50	15.00	17.50	20.00	22.50

TABLE 10

Crossing over values and map distances indicated by ordinates erected at different points on abscissa in figure 4.

POINT ON ABSCISSA	PERCENTAGE CROSSING OVER		MAP DISTANCE		TOTAL MAP DISTANCE
	Ga — Su	*Ga — de_1*	*Ga — Su*	*Ga — de_1*	
3.7	19.3	22.7	20.9	25.0	45.9
3.8	19.8	23.1	21.5	25.5	47.0
3.9	20.3	23.5	22.1	26.0	48.1
4.0	20.8	23.9	22.7	26.5	49.2
4.1	21.2	24.3	23.2	27.0	50.2
4.2	21.5	24.7	23.6	27.5	51.1
4.3	21.8	25.0	23.9	27.9	51.8
4.4	22.1	25.3	24.2	28.3	52.5

The map distance between *Su* and de_1 is 50 units as determined from HALDANE'S table (HALDANE 1919). These tables are, of course, based on the results from the sex chromosome in Drosophila but since they have been found to apply equally well to the other chromosomes of Drosophila and to the linkage groups in Primula, it is probable that they hold for the linkage groups in maize.

The problem in this case is to determine at what point on the abscissa in figure 4 an ordinate can be erected which crosses the two curves at points, whose values converted into map distances and combined, equal 50 units. Table 10 shows the results of erecting ordinates at a number of points along the abscissa.

It is noted that an ordinate erected at 4.1 crosses the two curves at points whose values best fit the facts. In other words, if 4.1 times as many *Ga* as *ga* gametes accomplish fertilization, then the crossing over between *Ga* and *Su* is 21.2 percent and between *Ga* and de_1, 24.3 percent. The map distance between *Ga* and *Su* would then be 23.2 units and between *Ga* and de_1, 27.0 units. These two values combined equal 50.2 units which is approximately the map distance between *Su* and de_1.

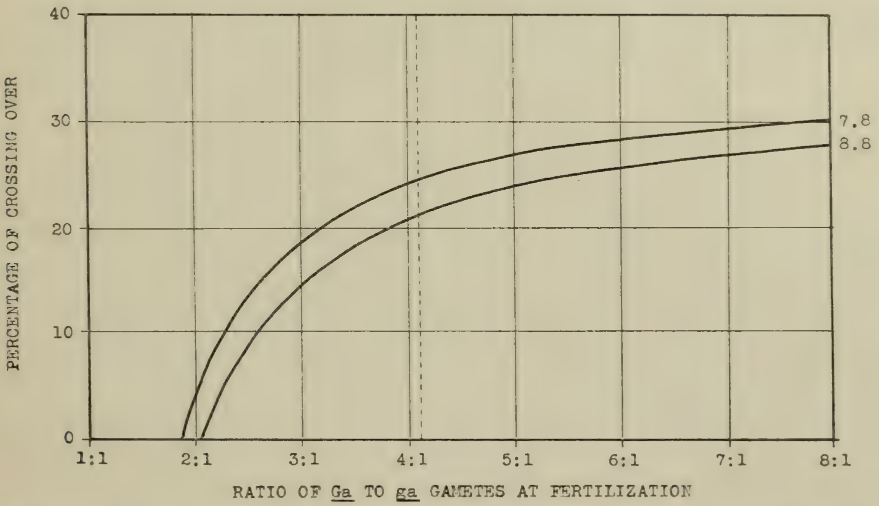

FIGURE 4. The various combinations of crossing over and relative effectiveness of two kinds of gametes which might cause deviations of 7.8 and 8.8 percent from the expected 25 percent recessives. An ordinate erected at 4.1 crosses the two curves at points whose values converted into map distances and added, equal the map distance between de_1 and *Su*.

It is realized, of course, that it is impossible to arrive at more than a crude approximation by this method. It is difficult to determine the true value of either of these influences until one of them is more accurately known. Furthermore, the assumption has been made that the relative proportion of *Ga* and *ga* gametes which accomplish fertilization is always the same. This is probably not strictly true. The data already given, and further data from mixed pollinations presented later, indicate that the selective action is due to differential pollen tube growth. The ratio of the two classes of gametes at fertilization would, therefore, depend to some extent on length of styles, weather conditions, and perhaps many other factors. In the long run these various influences would be expected to balance each other and over a period of years the ratio of the two types of gametes would be relatively constant. In comparing two series of observations, however, a variation in the relative effectiveness of the *Ga* and *ga* gametes must be kept in mind.

PREDICTING THE SIZE OF THE DEVIATIONS

If these approximations have any value it should now be possible to predict the deviations which will occur when other characters on the sugary chromosome are crossed with Rice Pop, providing that the amount of crossing over between sugary and the new character is known. A recent paper by WENTZ (1925) includes data which answer this purpose admirably. WENTZ found that a defective seed type de_{su} is linked with sugary with 3.2 percent crossing over. Since the crossing over between the *Ga* and *Su* loci is approximately 21.2 percent, then the crossing over between *Ga* and de_{su} should be about 23.8 or 18.6 percent depending on the direction of the de_{su} gene from the sugary locus. With the *Ga* and *ga* gametes occurring at fertilization in the ratio of 4 to 1, a cross between Rice Pop (*GaDe GaDe*) and WENTZ's defective (*gaDe gade*) should produce either 17.1 or 15.6 percent recessives on the segregating ears. Fortunately, WENTZ has crossed his defective seed strain with a stock tracing back to Rice Pop ancestry and apparently heterozygous for the accessory factor of the Pop parent. Eight segregating ears in the F_2 endosperm generation of this cross gave an average of 19.8 percent defective seeds as is shown in table 1 of WENTZ's paper. This value differs from the value predicted above by 2.7 percent.

Through the kindness of Professor WENTZ the detailed data on the individual ears of this cross, which were not included in his published paper, were made available to us. These data showed that three of the eight ears gave approximately normal ratios averaging 24.7 percent recessives. The other five ears, however, all deviated from a normal ratio by significant amounts and when combined gave a total of 1475 normal and 309 defective seeds, an average of 17.3 percent recessives.

The agreement of this value with the 17.1 percent recessives predicted by the above calculations is surprisingly good and is either a remarkable coincidence or an excellent bit of evidence in substantiation of our hypothesis.

The position of the factors on the sugary chromosome is, then, approximately as shown in one or the other of the two following diagrams.

$$de_1 \qquad Ga \qquad Su \quad de_{su} \quad Tu$$

$$de_{su} \quad Su \qquad Ga \quad Tu \quad de_1$$

Which of these arrangements is the correct one will be determined when the character *tunicate ear* is crossed with Rice Pop. The crossing over between *su* and *Tu* is approximately 28.6 percent (EYSTER 1921). In the first arrangement *Tu* is so far from *Ga* that its segregation would be affected only very slightly by the differential pollen tube growth. In the second arrangement *Tu* is fairly close to *Ga* and if this is the correct one, marked deviations should occur in the tunicate ratios when this character is crossed with Rice Pop or other stocks carrying the *Ga* factor.

GAMETOPHYTE FACTORS AND MIXED POLLINATIONS

The presence in Rice Pop of a gametophyte factor which influences the rate of pollen tube growth in sporophytic tissue of the same genetic constitution, probably accounts to a large extent for the results obtained in mixed pollination experiments. (JONES 1920, 1922). In these tests where a mixture of the white pop type and a yellow seeded sugary type was applied to the silks of both, there was a marked preference of the plants for their own kind of pollen. The results of five such mixed pollinations are shown in table 11.

TABLE 11

The amount of selective action shown by maize in five pollen mixtures.

POLLEN MIXTURE NO.	NUMBER OF SEEDS				TOTAL NO. OF SEEDS	DEVIATION FROM PERFECT PROPORTION IN PERCENT
	A×A	A×B	B×A	B×B		
1	811	11	381	2006	3209	41.35
2	4222	27	466	1404	6119	37.22
3	1568	2	319	224	2113	20.56
4	1930	29	73	309	2341	39.71
5	4084	6	963	290	5343	11.50

If there were no differences in pollinating ability, the proportion should be a perfect one within the limits of random sampling. The white smooth-seeded plants are designated as A in the tables and the plants with yellow, sugary seeds are listed under the heading of B. Without selective action the ratio of crossed to selfed seeds should be the same on ears of both types, regardless of the amount or viability of each kind of pollen. The last column gives the deviations from the closest calculated perfect proportion based upon the percentage of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation of 50 percent would indicate complete functioning of each kind of pollen on its own flowers to the exclusion of the foreign type. The deviations actually range from 14 to 41 in favor of self-fertilization. In some tests the deviations have been even higher but only the above five groups are given here because these were also used in determining differences in pollen tube growth.

That the selective action is due, in part at least, to differences in pollen tube growth, is indicated by the data in table 12. The ears were arbitrarily divided into top and bottom halves before shelling. The distance that the pollen tubes had to travel differed considerably in the case of seeds produced at the tip of the spike as compared with those at the base. If the plant's own pollen tubes grew more rapidly than the foreign tubes, we would expect fewer cross-fertilized seeds at the base than at the tip of the spike.

TABLE 12

The ratio of crossed seeds in upper and lower halves of ears resulting from mixed pollinations.

POLLEN MIXTURE NUMBER	PLANT TYPE	TOTAL NO. OF SEEDS	NO. OF CROSSED SEEDS PER 100		RATIO OF CROSSED SEEDS TOP : BOTTOM
			TOP	BOTTOM	
1	Pop	822	1.99	.54	3.7 : 1
1	Sugary	2387	17.23	14.64	1.1 : 1
2	Pop	4249	1.22	.05	24.4 : 1
2	Sugary	1870	26.66	23.01	1.2 : 1
3	Pop	1570	.25	.00	.25 : 0
3	Sugary	543	60.07	57.14	1.1 : 1
4	Pop	1959	2.54	.32	7.9 : 1
4	Sugary	382	24.73	13.78	1.8 : 1
5	Pop	4090	.10	.20	.5 : 1
5	Sugary	1253	78.43	75.35	1.1 : 1
Total and	Pop	12,690	1.22	.22	5.6 : 1
Average	Sugary	6,435	41.42	36.78	1.1 : 1

The last column in table 12 shows the ratio of crossed seeds in the top halves to those in the bottom halves. In all but one mixture there was a greater proportion of crossed seeds in the top halves of the ears and in this one exception there were only six crossed seeds in a total of four thousand. It is also noted that the excess of crossed seeds in the upper part of the inflorescence is much greater on ears of the pop type than those of the sugary type. In the former there were 5.6 times as many crossed seeds in the top halves while in the sugary ears there were only 1.1 times as many crossed individuals in the upper part of the inflorescences. The marked selective action found in these mixtures is apparently due to a condition in the pop parent which permits the plant's own pollen tubes to grow more rapidly in its own styles. That this condition is brought about by a gametophyte factor on the sugary chromosome has already been indicated.

It will be noted that the data also indicate a slight preference of the sugary plants for their own kind of pollen. Probably there are many genetic factors which affect pollen tube growth and any of these which cause increased growth when the sporophytic tissue is of the same genetic constitution, will give the plants' own pollen an advantage over foreign pollen. Almost all pollen mixtures show a slight selective action in favor of the plants' own pollen but only where one of the types possesses a factor which causes a marked increase in the rate of pollen tube growth, such as is found in Rice Pop, is the selective action so decided.

DISTORTED RATIOS IN WAXY ENDOSPERM

The deficiency of waxy seeds originally noted by COLLINS and KEMPTON (1911) in the F_2 endosperm generation of hybrids between waxy and other varieties of maize can also be explained, at least partially, by the action of one or more gametophyte factors linked with the *Wx wx* pair.

New interest in the inheritance of waxy has recently been aroused by the discovery that the carbohydrate reserve in the waxy endosperm is of a different chemical nature than that of other varieties of maize, staining red with iodine, while other types of endosperm stain a deep blue (WEATHERWAX 1922), and that this difference in reaction to staining is also apparent in the pollen grains (BRINK and MACGILLIVRAY 1924, DEMEREC 1924, LONGLEY 1924) and in the embryo sac. (BRINK 1925, KIESSELBACH and PETERSEN 1926)

KIESSELBACH and PETERSEN (1926) have summarized all the available data on the inheritance of waxy endosperm, including considerable new data of their own. When all the results are combined there is a deficiency of 1.1 percent from the expected 25 percent when the heterozygote is selfed and .7 percent from the expected 50 percent when segregating pollen from heterozygous plants is applied to the pure recessives. These writers conclude that "the evidence at hand seems insufficient to definitely establish the causes or significance of the deviations", although the deviation in the first case is 14.8 times the probable error and in the second 5.4 times the error.

The difficulty of interpreting the waxy situation is not due to a deficiency of data but to the fact that data, which are not strictly comparable, have been combined. Different investigators have used different stocks in crossing with waxy, and the waxy stock itself after repeated crossings and extractions, since it was first brought from China, is probably far from homogeneous. If accessory factors are involved in the inheritance of waxy it will be readily seen that the procedure of combining data from a wide variety of sources and especially that of combining different generations, is not a sound one. Merely adding a few thousand individuals to the third of a million already counted will contribute very little toward solving the cause of the deviations.

The fallacy of combining the data from separate generations is well illustrated by the defective seed ratios in table 3. An F_2 ear with 32.4 percent recessives produced F_3 progenies with an average of 23.8 percent recessives. At first glance it appeared that the deviation in F_2 was not inherited. Closer examination, however, shows that although the average percentage of defective seeds in F_3 is almost normal, the individual progenies show marked deviations, some in one direction, some in another.

ANALYSIS OF KEMPTON'S DATA

That a somewhat similar situation exists in the waxy stocks is shown by data from KEMPTON (1919). KEMPTON'S F_2 progenies gave an average of 23.7 percent waxy; his F_3 progenies, an average of 24.6 percent waxy. The difference between these two generations is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials.

The difference between the second and third generations cannot be explained by consistent errors in classification or to regular differences in viability, rate of pollen tube growth, or resistance to unfavorable influences inherent to the waxy factor *itself*, since all of these influences would produce as great a disturbance in one generation as in another. The situation, however, is exactly what would be expected if an accessory factor linked with the waxy gene is involved.

Further evidence of the action of an accessory factor influencing the waxy ratio is found in the data in table 1 of KEMPTON'S paper (1919). In this table are given all the results of selfing and intercrossing F_1 plants. Ordinarily, of course, these two types of pollination should give identical results but if an accessory factor is present in part of the plants, differences might well occur. KEMPTON, in intercrossing a pair of F_1 plants, frequently also self-pollinated one or both members of the pair and the data resulting from selfing are, fortunately, included in the table and can be compared to those obtained from the intercrossing.

A study of the pedigree numbers in this table show that there are 26 intercrosses in which the segregation of the two F_1 plants used in making the cross, has also been determined by selfing. Some of the F_1 plants gave almost normal ratios, others showed a deficiency of waxy seeds. Arbitrarily dividing the selfed plants into *normal* and *low*, using a deviation of twice the probable error as a basis, the intercrosses can be arranged into the following groups; *low* × *low*, *normal* × *low*. *low* × *normal*. and *normal* × *normal*.

Obviously, if the deviations in the selfed plants are merely due to chance, then grouping the intercrosses on the basis of the segregation in the selfed parental plants can have no effect on the average percentage of waxy in each group. If, however, the deviations are due to some condition inherent in the plants, it might be expected that *low* × *low* intercrosses would give different results than *normal* × *normal* pollinations. The results of grouping the intercrosses according to the behavior of both members of the parental pair are shown in table 13.

It is noted that the *low* by *normal* and *normal* by *normal* intercrosses are very closely alike, giving 25.1 and 25.4 percent of waxy seeds respectively. The *normal* by *low* and *low* by *low* intercrosses also resemble each other giving 22.9 and 22.6 percent of waxy seeds respectively. In other words, the groups of intercrosses in which pollen from *normal* plants was used gave *normal* ratios, the average for the two groups being 25.3 percent recessives, a very close approximation to the theoretical ratio. When pollen from *low* waxy plants was used, however, the intercrosses gave *low* waxy ratios, the average for the two groups being 22.7 percent waxy, a deviation from the theoretical ratio of 5.8 times the probable error.

It should be mentioned that in addition to the intercrosses shown in table 13 there are two involving plants with *high* waxy ratios. One of these, *normal* × *high*, gave 21.3 percent waxy, the other, *high* × *low*, 28.1 percent waxy. No conclusions regarding the significance or meaning of plus deviations can be drawn from these two ears.

It is apparent that the arbitrary grouping of the F_1 plants into *low* and *normal* on the basis of their deviations has actually divided them into classes which are inherently distinct. Plants giving *low* ratios when selfed give almost exactly the same ratios when their pollen is used on other plants. The remaining plants give ratios closely approximating *normal* whether selfed or intercrossed. In other words, *the random assortment of the male gametes at fertilization is disturbed in about half of the plants, and is relatively normal in the remainder.*

These facts can readily be explained by assuming that one of the parents of KEMPTON's hybrids was heterozygous for an accessory factor which affected the chances of the two kinds of gametes in accomplishing fertilization. Probably it was the waxy stock which brought in the accessory gene because deviations have been found in almost every case that waxy has been crossed with other stocks.

With the evidence of an accessory factor in mind it should be possible to isolate from such crosses as KEMPTON's, some heterozygous waxy lines which regularly give normal ratios, some which give a majority of minus deviations and others a majority of plus deviations. We have no doubt that such lines will soon be reported and that crosses with other characters on the waxy chromosome will indicate the approximate location of the accessory factor. BRINK (1925) has, in fact, evidence from one of his crosses which indicates the presence of an accessory factor closely linked with the *I* factor, which is located on the waxy chromosome.

The gametophyte factor in the waxy stock probably disturbs the ratios by causing differential pollen tube growth as does the factor in the sugary and defective seeds stocks. At least this is the simplest explanation and there is some evidence of its correctness from BRINK's (1925) experiments in which he found that fewer waxy seeds were produced on ears with long styles compared to those in which the styles were cut short.

PECULIAR RATIOS IN COLOR SEGREGATION

It is not improbable that COULTER's aberrant segregation in a stock heterozygous for the *C* factor (COULTER 1925) is due to the same accessory factor which affects the waxy ratios, since *C* and *wx* are members of the same linkage group.

COULTER's stock produced some progenies with marked minus deviations of white seeds, some with marked plus deviations, and others with normal ratios. He explains the deviations on the basis of linkage with a zygotic lethal factor and it is true that they can be fairly satisfactorily explained on this basis, although some of his *high* white ratios are higher than would be expected. Zygotic lethals, even with complete linkage, (repulsion phase) should cause a distortion of the ratio only from a 3 : 1 to a 2 : 1. A large proportion of COULTER's high ears average higher than 33.3 percent recessives and a number of them show 38 to 40 percent of white seeds.

Furthermore, zygotic lethals in maize are usually expressed in the form of defective seeds, and when these are present they can be readily noted and their numbers accurately determined. COULTER asserts that aborted seeds are sometimes found in this stock but apparently not regularly. He writes[1] "Many of my ears have the random distribution of empty pericarps so characteristic of the defectives. On other ears it seems impossible to detect the presence of the abortive grains. Doubtless accessory factors are at play to determine the degree of expression of this character."

From the experience in studying the inheritance of fourteen types of defective seeds, (MANGELSDORF 1926), it may be said that when defective seeds are present at all, their segregation is usually clear-cut and their number can be readily determined. The accessory factor which COULTER postulates is probably not one which so affects the degree of expression of the defectives that they can no longer be distinguished from the normal seeds but rather a gametophyte factor which influences the ratio in which the defective seeds appear, as well as the segregation for color. If this is true it should be possible to isolate from COULTER'S stock, lines which give *high, normal* and *low* white progenies but which do not segregate for defective seeds, others which give *high, normal,* and *low* defective progenies but do not segregate for color.

HOW GAMETOPHYTE FACTORS OPERATE

If the gametophyte factor on the waxy chromosome is one which affects the rate of pollen tube growth, it differs in two respects from the *Ga* factor on the sugary-defective chromosome. The first difference is that the waxy pollen tube factors reduces the rate of pollen tube growth instead of stimulating it. The second difference is that it operates regardless of the genetic constitution of the sporophytic tissues in which the pollen tubes grow. This is shown by the fact that the *normal* × *low* intercrosses in table 13 gave exactly the same results as the *low* × *low*.

There is, of course, no reason for believing that all gametophyte factors behave in the same way. It is probable that factors operating in the gametophyte generation may have their effect in many different ways, for example; in causing differential pollen tube growth, duration of viability, resistance to drying out, excessive moisture, high and low temperature, etc. Even the factors affecting the rate of pollen tube growth alone may fall into at least four distinct categories, as follows:

1. Factors which reduce the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.

2. Factors which reduce the rate of pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

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[1] In a letter.

3. Factors which stimulate the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.

4. Factors which stimulate pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

TABLE 13

Kempton's F_1 intercrosses grouped on the basis of the segregation in the selfed parental plants.

TYPE OF INTERCROSS	PEDIGREE NUMBERS			PERCENTAGE WAXY		
	♀	♂	INTERCROSS	♀	♂	INTERCROSS
L×L	1534	1743	1535	21.7	20.5	22.7
L×L	1543	1723	1542	20.9	21.0	23.3
L×L	1723	1543	1722	21.0	20.9	23.9
L×L	1743	1534	1742	20.5	21.7	21.3
Average						22.9
N×L	1117	1118	1116	24.2	21.9	26.0
N×L	1515	1726	1518	25.0	21.6	21.5
N×L	1129	1135	1130	24.1	21.5	21.0
N×L	1133	1118	1134	24.6	21.9	16.4
N×L	1137	1135	1138	26.0	21.5	25.1
N×L	1741	1551	1740	22.7	21.2	25.6
Average						22.6
L×N	1111	1132	1110	22.2	23.3	27.5
L×N	1118	1108	1119	21.9	26.4	25.8
L×N	1121	1117	1120	22.5	24.2	22.1
L×N	1551	1741	1550	21.2	22.7	20.6
L×N	1135	1137	1136	21.5	26.0	28.6
L×N	1726	1519	1725	21.6	23.8	24.2
Average						25.1
N×N	1105	1108	1104	26.4	26.4	23.7
N×N	1527	1745	1526	23.3	23.8	26.8
N×N	1549	1749	1548	24.6	26.3	24.0
N×N	1128	1125	1127	25.0	24.0	24.4
N×N	1132	1117	1131	23.3	24.2	22.9
N×N	1721	1515	1720	23.2	25.0	30.7
N×N	1745	1527	1744	23.8	23.3	26.0
N×N	1749	1549	1748	26.3	24.6	25.8
Average						25.4

Gametophyte factors which fall into the second and third of these categories have apparently already been found in maize in the waxy and sugary-defective pollen tube factors. An example of the first group is found in the inheritance of self-sterile classes in Nicotiana (EAST and A. J. MANGELSDORF 1925, 1926) where certain classes are almost completely eliminated presumably because of the slower rate of pollen tube growth.

CARBOHYDRATE METABOLISM AND POLLEN TUBE GROWTH

It may have been noted that all the characters so far reported *su*, *wx*, de_1 and de_{su} which appear to be associated with differences in pollen tube growth, are endosperm characters which are also associated with differences in the nature or amount of carbohydrate storage.

BRINK (1925) is of the opinion that differential pollen tube growth operates in combination with, or is caused by, differential carbohydrate metabolism. The fact that these four above mentioned characters do affect the nature or amount of carbohydrate storage might be considered as evidence in favor of such a view. We are of the opinion, however, that this is merely a coincidence; that distorted ratios in maize have been found mainly in endosperm characters only because such characters have been studied on a larger scale than any others. We feel certain that similar disturbances in the ratios of seedling and plant characters will also be found when these are studied on a greater scale. In fact, *high*, *low*, and *normal* ratios, apparently due to differential pollen tube growth, are now being obtained in a white seedling stock. (MANGELSDORF Unpublished).

The possibility, however, that differential carbohydrate metabolism may have some effect on pollen tube growth and that certain sporophytic characters may *per se* affect the rate of growth, is not denied. The marked deficiency of waxy seeds which BRINK (1925) obtained in a heterozygous waxy, homozygous sugary stock cannot, perhaps, be explained on the basis of gametophyte factors alone.

DISCUSSION

Selective fertilization has always offered an attractive explanation of peculiar results for which it was difficult to account by other means, and has frequently been called into account for unexpected departures from Mendelian ratios. Cuenot's yellow mice, CORREN'S aberrant ratíos in Melandrium, HERIBERT-NILLSON'S unusual segregation of certain characters in *Oenothera*, are examples[1].

EAST (1922) includes as one of the "Provisional Laws of Heredity" the statement that "there is no selective fertilization between complementary, compatible, functional gametes". This is probably true if the term

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[1] BRIEGER (1926) has recently summarized the cases in which selective fertilization might be used to interpret the results.

"fertilization" is confined to the actual fusion of the nuclei and a distinction is made between selective fertilization and certain other influences which disturb the random assortment of the gametes at the time of fertilization. For example; in plants or animals in which free-swimming, motile sperm come in contact with the egg in large numbers, there may be no discrimination by the female gametes in favor of certain of the male gametes, providing that all are compatible. There is no reason for believing, however, that all the sperm, in all cases, are equally capable of reaching the egg. It is possible that some classes are so handicapped as a result of their chromosomal constitution that though produced in full quota at gametogenesis, they are in minority at the time of fertilization.

In angiosperms an entire generation elapses between the time of gametogenesis and fertilization and the opportunity for differential elimination of certain classes is relatively large.

The pollen tube of angiosperms represents the remnant of the elaborate gametophyte generation in lower plants, in many of which this generation is of equal or greater importance than the sporophyte. In such plants there can be no question that the hereditary factors have almost, if not quite, as great an influence in the gametophyte as in the sporophyte generation. In fact, characters which have their expression in the gametophyte generation have recently been reported by WETTSTEIN (1924) in the mosses and ALLEN (1925) in the liverworts. In angiosperms the gametophyte generation has become greatly reduced and the hereditary factors which influence it have, no doubt, been correspondingly lessened, though not entirely eliminated.

Mendelian factors have been found to affect practically all stages in the ontogeny of the maize plant. The growing embryo, the endosperm, the resting stage of the seed, the young seedling, stature, chlorophyll development and reproductive processes, are all governed by genetic factors which may be unlike for different individuals. *It would be strange indeed if the gametophyte generation, brief though it has become, should be entirely deprived of the governing influence of various Mendelian factors.*

It is not surprising, therefore, to find in maize several Mendelian factors which have their expression in the gametophyte generation. These factors produce a definite physiological effect upon the gametophytes in which they occur, speeding up development in one case, reducing it in the other. They are transmitted from generation to generation in the same manner as any other Mendelian factors, occupy definite loci on certain chromosomes and show characteristic linkage and crossing over phenomena.

In general, the genetic factors which affect the gametophyte probably have little influence on ordinary Mendelian ratios in the sporophyte. In the female gametophyte, at least in angiosperms, they probably seldom cause any disturbance. In the male gametophytes they can have no effect as long as the parental sporophyte remains homozygous for the factors which influence the gemetophyte generation. Even when the gametophytes differ genetically, the favorable and unfavorable factors would, in the long run, tend to balance each other and no classes of gametes would be given a decided advantage. Only in occasional instances where a gametophyte factor produces a marked effect and is at the same time closely linked with a recognizable character in the sporophyte, is there any disturbance in the Mendelian ratios. Even here the total disturbance may not be noticeable when data from several generations are combined because a reversal of the linkage relations will cause deviations to balance each other.

Genetic factors affecting the gametophyte generation, giving certain gametes an advantage or handicap in accomplishing fertilization, need not contradict the Mendelian conception of random assortment. In some plants, however, they must be taken into consideration, as are linkage and lethal factors, in accounting for unexpected results.

SUMMARY

1. A stock segregating for defective seeds, de_1, regularly produces *high* ears with approximately 33 percent recessives, *normal* ears with 25 percent recessives, and *low* ears with 17 percent recessives.

2. These results are explained by assuming a factor, *Ga*, which has its expression in the gametophyte generation, stimulating the rate of pollen tube growth. *Ga* is linked with de_1 and in the coupling phase causes an excess of recessives; in the repulsion phase, a deficiency.

3. The cross of sugary × Rice Pop indicates the presence of a similar gametophyte factor linked with *Su* in the Rice Pop parent. The percentage of sugary seeds in F_2 is 16.2.

4. *Su* and de_1 are linked, with 39 percent crossing over, and it is probable that the gametophyte factor in both stocks is the same.

5. On this assumption it has been calculated that the *Ga* gametes accomplish fertilization 4.1 times as frequently as *ga* gametes and that the crossing over between *Ga* and de_1 is 24.3 percent; between *Ga* and *Su*, 21.2 percent.

6. That these values are approximately correct is indicated by WENTZ' data from a cross between de_{su}, a fourth character in this group, and a

stock with Rice Pop ancestry. This cross resulted in deviations approaching those predicted by the above calculations.

7. Gametophyte factors probably account, to a large extent, for the peculiar results obtained in mixed pollination experiments.

8. An analysis of Kempton's data indicates the presence, on the waxy chromosome, of a gametophyte factor causing distortions in the starchy: waxy ratio.

9. The action of the same factor is suggested as explanation of Coulter's peculiar ratios in aleurone color segregation.

10. The various ways in which gametophyte factors may operate and their effects on Mendelian ratios in the sporophyte are discussed in detail.

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Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879.

CYTOLOGICAL AND GENETIC STUDIES ON FRAGARIA

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Reprinted from GENETICS 11:590–604, November, 1926

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Received May 21, 1926

TABLE OF CONTENTS

PAGE
INTRODUCTION.... 590
Methods.... 592
Chromosome details.... 592
  a. Chromosomes of the European type.... 592
  b. Chromosomes of the Haut-bois type.... 593
  c. Chromosomes of the American type.... 593
  d. Chromosomes of the Chilean type.... 593
  e. Chromosomes of the Cultivated-variety type.... 593
  d. Chromosomes in F_1 hybrids and F_2 hybrids.... 596
DISCUSSION.... 597
  a. Polyploidy in the Fragaria species.... 597
  b. Species hybrids.... 600
SUMMARY.... 601
LITERATURE CITED.... 602

INTRODUCTION[1]

The eleven species and twelve cultivated varieties of the genus Fragaria which were included in this investigation may be divided into five different groups:

Group 1.—The European type, represented typically by the species *F. vesca* which is the most common wild species in Europe and is also widely distributed over NORTH AMERICA and western South America.

Fragaria vesca L.
F. americana Britton.

---

[1] Several genetic investigations on the genus Fragaria were started at the BUSSEY INSTITUTION in 1921 with material furnished by Mr. G. M. DARROW of the UNITED STATES DEPARTMENT OF AGRICULTURE. Mr. DARROW has very kindly visited the laboratory several times, has identified and checked the living material, and has made many suggestions of great value during the progress of the work

In 1924, Mr. K. ICHIJIMA undertook to make a cytological examination of all of the pure species and of the most interesting hybrids then on hand. This study he finished in the spring of 1925, but was unable to make a detailed report at that time because of a long illness. In the winter of 1925–1926, however, the present paper was written, and left to the undersigned to edit.

In the meantime, a paper entitled "Chromosomes and their significance in strawberry classification," by Dr. A. E. LONGLEY, was published in the Journal of Agricultural Research 32: 559–568, issued March 15, 1926. The data reported here corroborate many of Dr. LONGLEY's results and furnish certain additional facts. Both papers gain in value from having been written simultaneously and independently. E. M. EAST

F. californica Cham. & Schlecht.
F. mexicana Schlecht.
F. Helleri Holz.
F. bracteata Heller.

Group 2.—The Haut-bois type, represented by *F. elatior*, indigenous to Europe.

Fragaria elatior Ehrh.

Group 3.—The American type, represented typically by the species *F. virginiana* which is very widely distributed in North America.

Fragaria virginiana Duchesne.
F. glauca Rydb.

Group 4.—The Chilean type, indigenous to the Pacific Coast of North and South America and represented by the species *F. chiloensis*.

Fragaria chiloensis Duchesne.
F. cuneifolia Nutt? from Oregon.

Group 5.—Certain cultivated varieties of a type often described as *F. grandiflora* in the Taxonomy of the genus.

Varieties used:

William Belt
Champion Early
New York

La Pearl
Clark's Seedling
Doctor Burrell
Chesapeake

Ettersburg
Gardners
Progressive
Success

In addition to the above, the following hybrids were examined:

F₁ hybrids between the species—

F. vesca ♀ × *F. Helleri* ♂
F. vesca ♀ × *F. americana* ♂
F. Helleri ♀ × *F. americana* ♂
F. bracteata ♀ × *F. Helleri* ♂ . . . . (type a)
F. bracteata ♀ × *F. Helleri* ♂ . . . . (type b)
F. glauca ♀ × *F. virginiana* ♂
F. bracteata ♀ × *F. virginiana* ♂
F. mexicana ♀ × *F. virginiana* ♂
F. bracteata ♀ × *F. glauca* ♂
F. americana ♀ × *F. glauca* ♂
F. grandiflora var. *Dunlap* ♀ × *F. platypetala* ♂

RYDBERG'S (1912) descriptions have been followed in making up the above list. The species which he designates as *F. Helleri*, however, is merely a pink-flowered variety of *F. vesca*.

METHODS

Maturation divisions occur very early in Fragaria flowers, and the chromosomes are very minute. For these reasons it was necessary to modify the cytological methods ordinarily in use in several particulars.

Flower buds were collected when about 1 mm in diameter, the calyx stripped off and fixed for 12 hours. For somatic chromosome counts, very young, thick root-tips from newly produced runners were found to be the most suitable material. As fixatives, Flemming's medium solution and chrom-acetic acid were found to be unsatisfactory. Bouin's solution as modified by ALLEN (1916) gave much better results, except in the hybrid between *F. bracteata* and *F. Helleri*, where straight Bouin's solution was used.

The material was washed 5 hours in water and then moved up through the alcohols. It was found necessary to transfer slowly to xylol, keeping the material 4 hours each in various percentages beginning with 15 percent.

The paraffine blocks cut very satisfactorily and the material examined unstained was not shrunken. But if care was not taken in staining, shrinkage was quite marked.

The best method of staining was found to be the use of 4 percent iron-alum as a mordant for only 2 hours, followed by transfer to haemotoxylin for 3 hours. No destaining was necessary.

CHROMOSOME DETAILS

a. *Chromosomes in Group 1—the European type*

All of the six species of this group which were investigated have 7 pairs of chromosomes in the pollen mother cells. No irregular behavior of the chromosomes in the course of the heterotypic division was observed. In the earliest stage the pollen mother cells are closely packed in the anther cavity. As the meiotic division proceeds the cells gradually begin to separate from one another. After the synapsis there is generally a distinct spireme stage in which the double thread-like nature can clearly be seen; but no second contraction has been observed. At early diakinesis the paired chromosomes are often unequal in size (figure 1), but gradually contract into a uniform size in the later stage. At this stage the 7 pairs of chromosomes can be seen clearly (figure 2). When the paired chromosomes become almost spherical the nucleolus and nuclear membrane disappear. The chromosomes then gather toward the center and arrange themselves on the equatorial plate. It is here at the metaphase and at the late anaphase that the chromosomes can be most readily counted (figures

3, 4). After the telophase and the subsequent resting stage the cells begin the homeotypic division which is carried out in the regular manner (figure 5), the resulting tetrads being quite normal. The 14 somatic chromosomes are seen clearly and may be counted readily at the metaphase of the root-tip cell (figure 6). The somatic chromosomes appear in a characteristic figure and are all separated from one another in well fixed preparations.

b. *Chromosomes in Group 2—The Haut-bois type*

In the single species, *F. elatior*, belonging to this group, the gametic number of chromosomes in the pollen mother cell is 21. Figure 7 shows the chromosomes in the heterotypic metaphase. There is no irregular behavior of the chromosomes during the whole process of maturation which indicates the stability of the species cytologically.

c. *Chromosomes in Group 3—The American type*

Two species of this type were investigated, *F. virginiana* and *F. glauca*. Both of them possess 28 chromosomes as the gametic number. Owing to the minute size of the pollen mother cell it is quite difficult to count the chromosomes when such large numbers are present. After a number of experiments it was found that counts could best be made in the late diakinesis of the heterotypic division (figure 8). The somatic chromosomes are closely and irregularly packed in the small root-tip cells. The somatic number of chromosomes could not be determined definitely although it appears to be approximately 56.

d. *Chromosomes in Group 4—The Chilean type*

F. chiloensis and *F. cuneifolia* both show 28 chromosomes as the gametic number. The behavior of the chromosomes is quite regular throughout both the heterotypic and the homeotypic divisions. No abnormalities in chromosomal behavior were found (figures 9, 10).

e. *Chromosomes in Group 5—The Cultivated variety type*

Twenty-eight pairs of chromosomes were in found the pollen mother cells of each of these varieties. Abundant pollen grains are produced and the process of reduction appears to be regular. There is little difference in the chromosome behavior between this type and the American type, both of which have the same number of the chromosomes (figure 11).

PLATE I

FIGURE 1.—The 7 paired chromosomes at diakinesis in *Fragaria vesca* var.

FIGURE 2.—A later stage of the same.

FIGURE 3.—Metaphase of heterotypic division.

FIGURE 4.—Late anaphase.

FIGURE 5.—Metaphase of homeotypic division.

FIGURE 6.—Metaphase of division in the root-tip cell (14 somatic chromosomes).

FIGURE 7.—Metaphase showing 21 pairs of chromosomes (*F. elatior*).

FIGURE 8.—Diakinesis showing 28 paired chromosomes (*F. virginiana*).

FIGURE 9.—The same stage in *F. chiloensis*.

FIGURE 10.—Homeotypic metaphase of the same.

FIGURE 11.—Diakinesis showing 28 paired chromosomes (a cultivated variety).

FIGURE 12.—Metaphase of the F_1 hybrid of *F. bracteata*×*F. Helleri*, showing two different groups of chromosomes.

FIGURE 13.—Diakinesis of the same (14 paired chromosomes).

FIGURE 14.—Homeotypic metaphase of the same (14 chromosomes).

FIGURE 15.—Root-tip cell of the F_2 plant of *F. bracteata*×*F. Helleri*, showing 28 somatic chromosomes.

FIGURE 16.—Diakinesis of reduction division of F_1 of *F. bracteata*×*F. virginiana* showing 7 bivalents and approximately 21 univalents.

FIGURE 17.—Metaphase of the same.

FIGURE 18.—Early metaphase of the same,—univalents lagging behind.

FIGURE 19.—Late anaphase of the same.

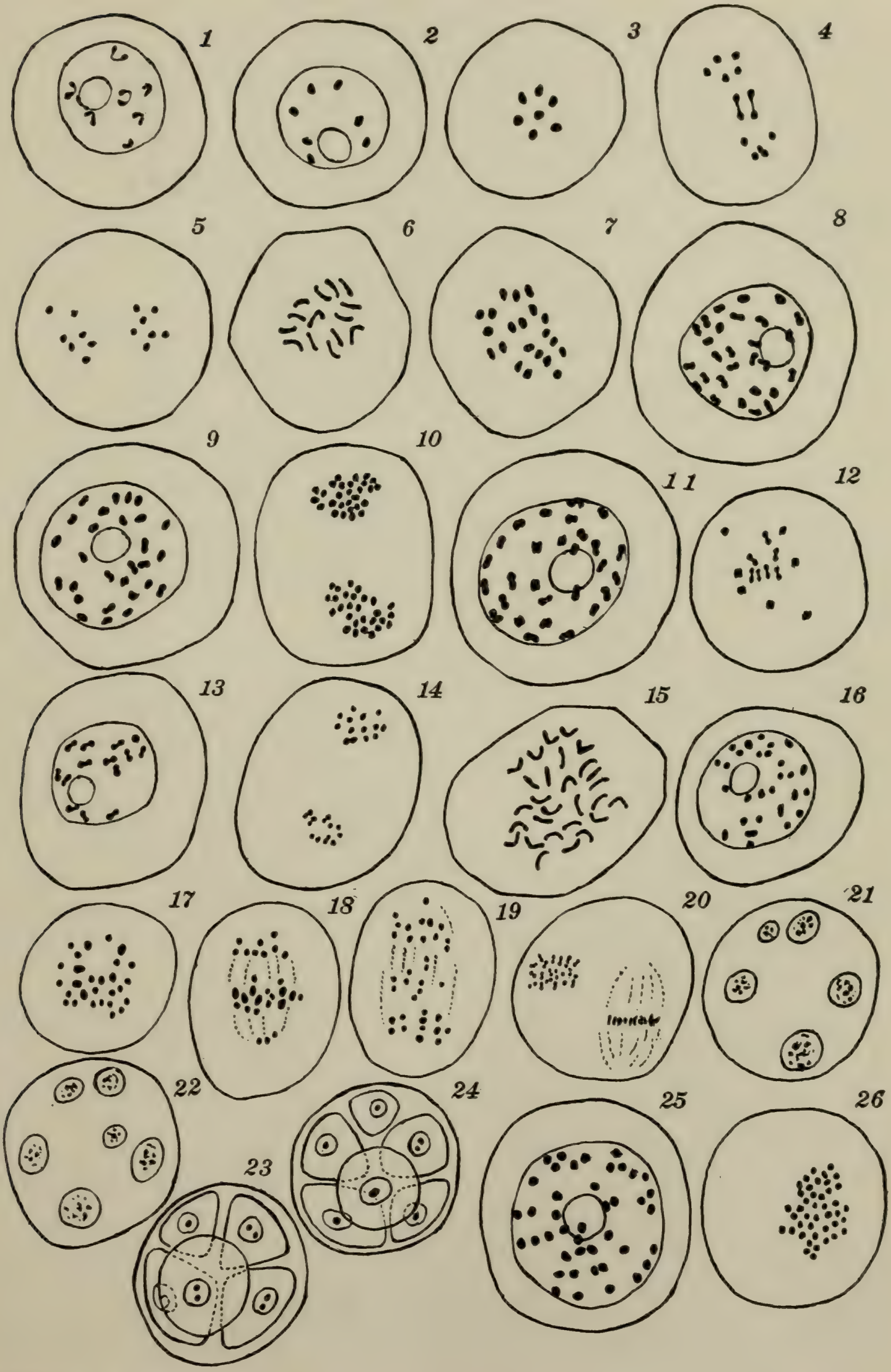
FIGURE 20.—Homeotypic metaphase of the same.

FIGURES 21–24.—Abnormal tetrad formations of the F_1 of *F. bracteata*×*F. virginiana*.

FIGURE 25.—Diakinesis in *Duchesnea indica*.

FIGURE 26.—Metaphase of the same.

Plate I



f. *Chromosomes in F_1 hybrids*

F_1 hybrids between species of the European type, such as *F. vesca*×*F. Helleri*, *F. vesca*×*F. americana*, *F. Helleri*×*F. americana*, and *F. bracteata* ×*F. Helleri* possess 7 pairs of chromosomes. Ordinarily there is no irregular behavior of the chromosomes, but an interesting exception has been found in the F_1 of one such cross. An individual in one of the populations of F_1 hybrids between *F. bracteata*×*F. Helleri* was found showing 14 pairs of chromosomes (figure 13) in the diakinesis of the pollen mother cell. Presumably the 7 chromosomes contributed by the bracteata parent and the 7 chromosomes contributed by the *Helleri* parent had doubled during some abortive attempt at cell division. Figure 12 shows one group of 7 chromosome pairs in the heterotypic metaphase which is easily distinguishable from a second group of 7 chromosome pairs. In the homeotypic division 14 chromosomes as a haploid number may be counted clearly (figure 14). The behavior of the gametic chromosomes seems to be normal. The F_2 hybrids obtained by selfing this plant showed 28 chromosomes in root-tip preparations (figure 15). The writer has not been able to investigate conditions in the pollen mother cells of the F_2 plants.

In F_1 hybrids between the species of the American type, for example *F. glauca*×*F. virginiana*, no abnormal chromosome behavior was observed. But hybrids between species of the different types, for example American type ($x=28$)×European type ($x=7$), which can be obtained only with difficulty, show many irregularities. Among a population of F_1 hybrids between *F. bracteata*×*F. virginiana* some individuals were found having 28 chromosomes in the heterotypic metaphase, of which 7, presumably bivalents, appear to be larger than the other 21 (figure 17). In the early metaphase there are approximately 28 chromosomes of which 7 bivalents are on the equatorial plate, while 21 univalents are found on the spindle (figure 18). Figure 19 shows 7 bivalents which, having divided, are pushing toward the poles, while the 21 univalents are lagging in their approach to the poles. It is obvious that each of the bivalents divides before moving to the poles, but the univalents pass at random to either pole without dividing. Owing to the minute size of the chromosomes, it is quite difficult to count the number of the bivalents and univalents in the heterotypic division. In figures 21–24 one may observe clearly the irregularity of the tetrad formation, a consequence to be expected from the previous behavior of the chromosomes. This irregularity of tetrad formation supplies the cytological basis of the practically complete sterility of these hybrids.

DISCUSSION

a. *Polyploidy in Fragaria species*

The foregoing investigation gives us another case of possible polyploidy in plant species. If the basic haploid chromosome number is taken to be 7, various Fragaria species may be interpreted as diploid, tetraploid, hexaploid and octaploid forms, that is, as forms having 7, 14, 21 and 28 pairs of chromosomes. Furthermore, *Duchesnea indica*, which once was classified as a species of Fragaria is found to have 42 pairs of chromosomes; and since it may be crossed with *Fragaria vesca*, it may possibly be regarded as a dodecaploid Fragaria (figures 25, 26).

The occurrence of chromosomes in a progressive arithmetical series has been found in several other genera. In Chrysanthemum TAHARA (1915, 1921) found species with haploid chromosome numbers 9, 18, 27, 36 and 45. In Viola, according to MIYAJI (1913) and MARCHAL (1920), there are species with 6, 10, 12, 24 and 36 haploid chromosomes. In Hieracium ROSENBERG (1917) found somatic numbers 18, 27, 36 and 54. In Crepis there are species with 6, 8, 10, 16, 18, 24 and 42 somatic chromosomes (ROSENBERG 1918, 1920). In Triticum SAKAMURA (1918, 1920) and SAX (1921, 1922) state that one species has 7 chromosome pairs, four species have 14 pairs, and three species have 21 pairs. In Campanula MARCHAL (1920) found 17, 34 and 51 haploid numbers. In Rubus LONGLEY (1924) found 7, 14 and 21 chromosome pairs. TÄCKHOLM (1920, 1922) and BLACKBURN and HARRISON (1921), investigating the genus Rosa, have established the most remarkable series with forms having 14, 21, 28, 35, 42 and 56 somatic chromosomes.

The European type of Fragaria which has the haploid chromosome number 7 may perhaps be regarded as the most primitive species of the genus. This species not only possesses the lowest chromosome number, but also its morphological characters appear to be the most primitive when compared with those of *F. elatior*, *F. virginiana*, and *F. chiloensis* which more closely resemble the horticultural varieties having higher chromosome numbers and more complex morphological characters. Furthermore the species of the European type are hermaphroditic, while certain species of the American type and of the Chilean type are partially diœcious.

No wild species were found possessing 14 pairs of chromosomes. Evidence of tetraploidy in Fragaria, therefore, rests upon a single case obtained in the cross between *F. bracteata* ($x=7$) and *F. Helleri* ($x=7$). The ordinary F_1 plants obtained from this cross were intermediate

PLATE II

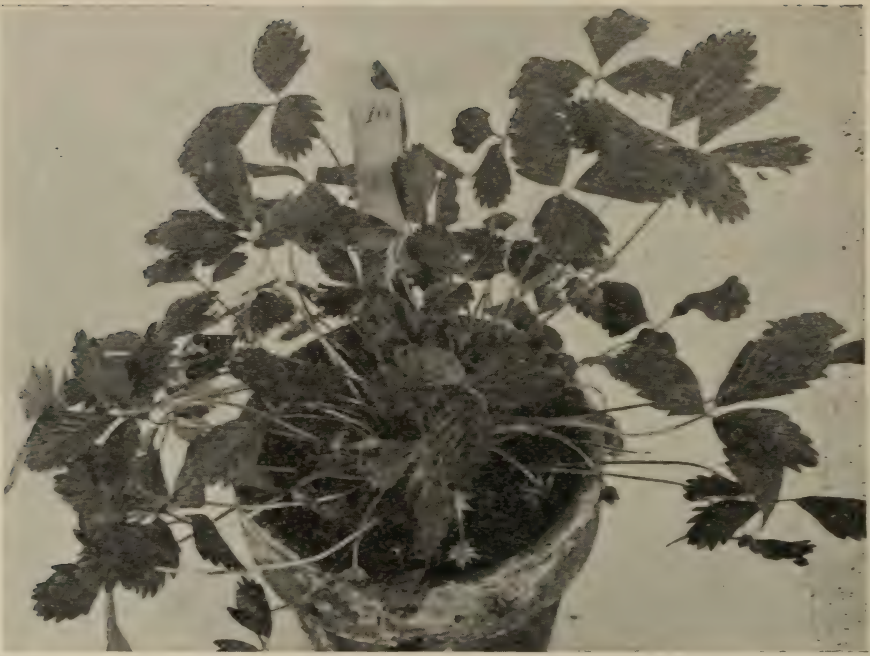


FIGURE A.—*F. bracteata.* A species of the European type.

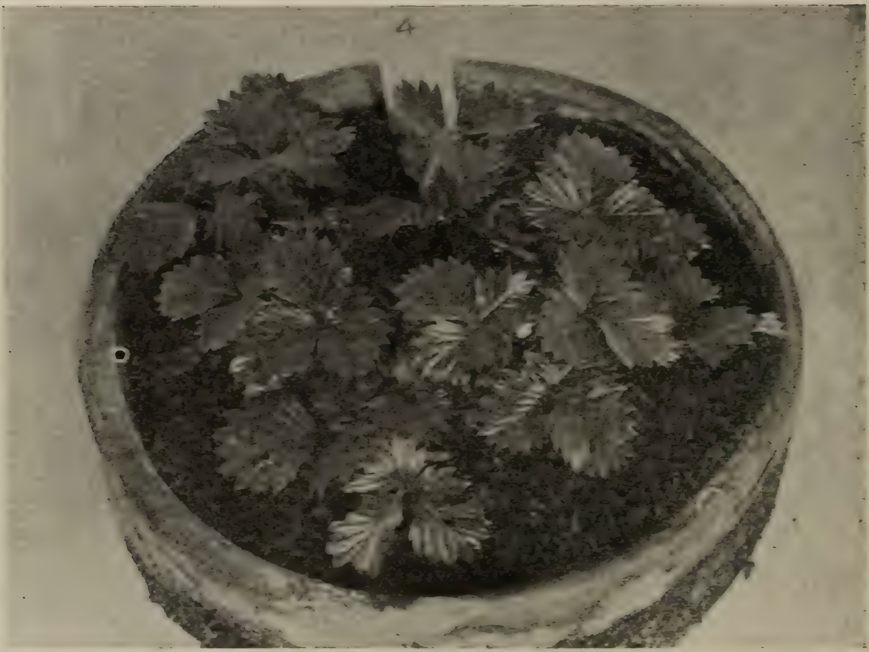


FIGURE B.—Hybrid, type b, of *F. bracteata*×*F. Helleri.*

PLATE III

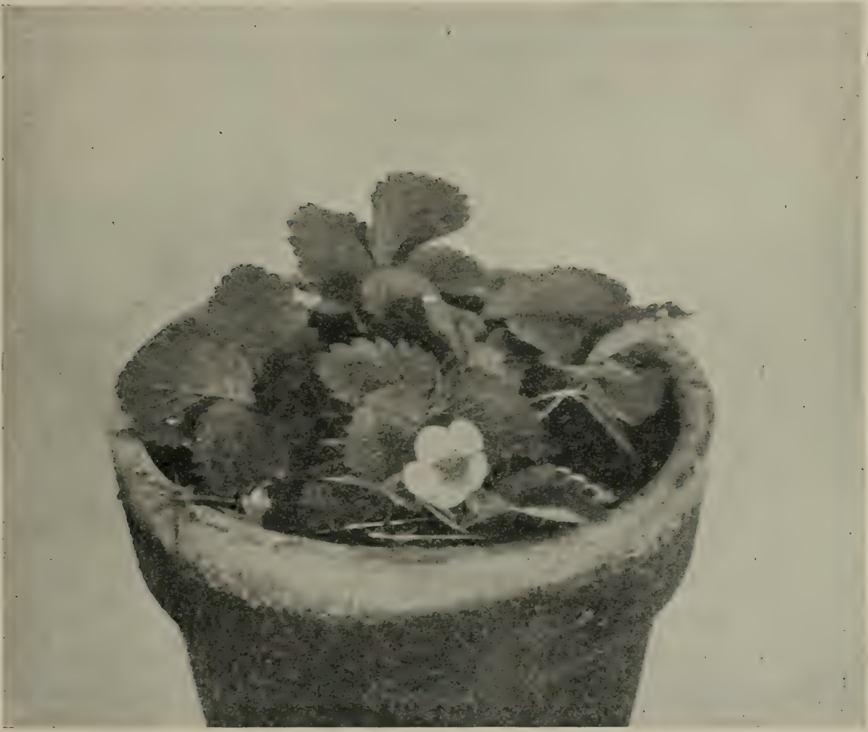


FIGURE A.—*F. virginiana*, a typical American type.

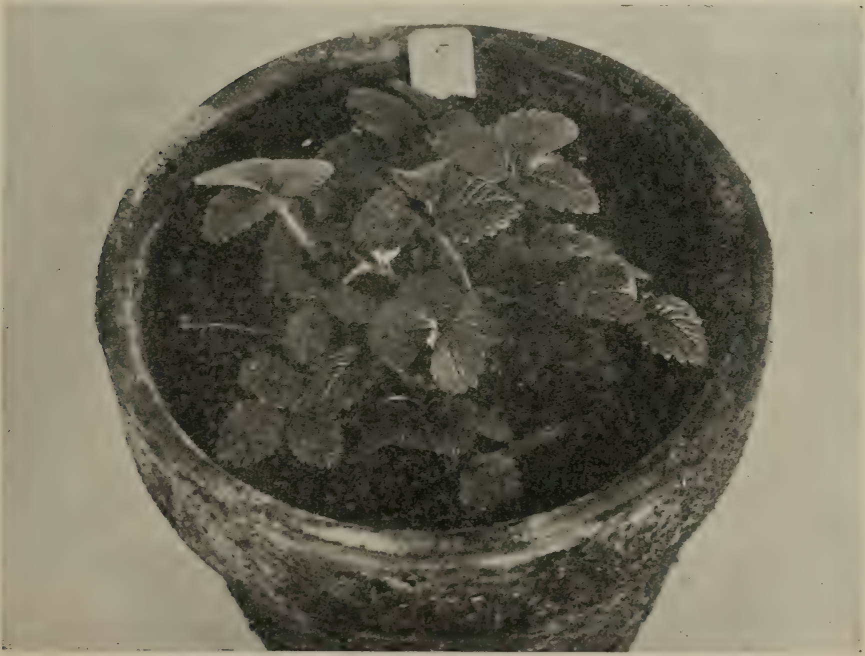


FIGURE B.—Hybrid of *F. bracteata*×*F. virginiana*.

between the parents except for flower color, the pink flower characteristic of *F. Helleri* being dominant. Nine of these ordinary hybrids (type a) were investigated cytologically and were found to have 7 pairs of chromosomes. The behavior at reduction was normal. The exceptional individual had 14 pairs of chromosomes, and instead of segregating and recombining the characteristics of the parental species as did the ordinary F_2 populations, it produced a uniform population of a new type. The leaves were larger, thicker and more crenate than either of the parents. The petals also were thicker and the pollen grains were decidedly larger than in the forms from which it arose (Plate 2, A and B). The root-tip cells of seven individuals were examined, and in each case 28 chromosomes were found. These plants may therefore be considered to be representatives of a new species.

Several other cases of plant tetraploidy which occurred under observation have been reported. *Oenothera gigas*, a mutant form of *O. Lamarckiana*, is a well known case (LUTZ 1907). In Primula DIGBY (1912) found a tetraploid form with 36 somatic chromosomes, and in Datura a tetraploid form has been found which has 48 somatic chromosomes instead of the 24 of the original species (BLAKESLEE, BELLING and FARNHAM 1920). In Nicotiana, CLAUSEN and GOODSPEED (1925) produced a true-breeding hybrid between glutinosa ($x=12$) and tabacum ($x=24$) which possessed 36 pairs of chromosomes.

b. *Species hybrids*

In the F_1 hybrids of *F. bracteata* ($x=7$) × *F. virginiana* ($x=28$), as previously stated, there are 7 bivalents and 21 univalents in the heterotypic spindle. In the reduction division the bivalents divide normally while the univalents pass at random, without dividing, to either pole. The behavior of these chromosomes in the second division has not been clearly observed. But the subsequent tetrad formation seems to be much disturbed, because the majority of the pollen mother cells form three, five or six daughter nuclei instead of the normal four, none of which are able to become normal mature pollen grains. The irregularities of the chromosome behavior show the cytological basis of the sterility of the hybrid. This view has been borne out in several other species hybrids (See Plate 3, A and B).

Similar chromosomal irregularities in the heterotypic division of hybrids have been investigated in many other species. In Drosera ROSENBERG (1909) has investigated a hybrid between a species with 10 haploid and a species with 20 haploid chromosomes which shows

10 bivalent and 10 univalent chromosomes at diakinesis. GATES (1909) found the chromosome number of the F_1 of certain Oenothera crosses to be 20 or 21, the sum of the gametic number of the parents. In an F_1 hybrid of *Hieracium auricula* ($x=9$)$\times$*H. aurantiacum* ($x=18$), 9 bivalents and 8 or 9 univalents were found in the reduction division (ROSENBERG 1917). In Triticum KIHARA (1919) described 14 bivalents and 7 univalents in the heterotypic prophase of the F_1 hybrid *T. polonicum* ($x=14$) $\times$*T. spelta* ($x=21$). YASUI (1921) found a similar chromosome behavior in Papaver hybrids. TÄCKHOLM (1920, 1922) found that species of the Canina section in Rosa have usually 7 bivalents with 14, 21 or 28 univalents in the reduction division. SAX (1922) reports that in the F_1 of *Triticum monococcum* ($x=7$)$\times$*T. turgidum* ($x=14$), there are 7 bivalents and 7 univalents in the heterotypic division; and the F_1 of the Emmer group ($x=14$)$\times$the Vulgare group ($x=21$) shows 14 bivalents with 7 univalents in the first meiotic division, resulting in microspores having from 14 to 21 chromosomes.

SUMMARY

1. The Fragaria species investigated may be divided according to their chromosome number into four groups having 7, 14, 21, and 28 chromosomes respectively.

2. F_1 hybrids between different representatives of the American type have 28 gametic chromosomes.

3. F_1 hybrids between different representatives of the European type have 7 gametic chromosomes, with the exception of the following case.

4. The cross of *F. bracteata*$\times$*F. Helleri* produced, in one case, a tetraploid form, having 14 gametic chromosomes, of which 7 are contributed by one parent and 7 by the other parent. The F_2 plants which were obtained by selfing this tetraploid form have 28 somatic chromosomes in the root-tip cells, and possess distinct morphological characteristics which have not been observed in any other species. The type may be regarded as a new species.

5. In the F_1 hybrid of *F. bracteata*$\times$*F. virginiana* 7 bivalents and approximately 21 univalents were found. The bivalents divide normally, but the univalents pass at random to either pole without dividing. The tetrad formation seems to be greatly disturbed, consequently there are no normal mature pollen grains found.

6. *Duchesnea indica* is found to have 42 gametic chromosomes; and judging from possibility of crossing with *F. vesca,* it may possibly be considered to be a dodecaploid form of Fragaria.

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GENETICS, NOVEMBER, 1926

TABLE OF CONTENTS

PAGE

STERN, CURT AND BRIDGES, CALVIN B., The mutants of the extreme left end of the second chromosome of *Drosophila melanogaster*.... 503

ZIRKLE, CONWAY, Some numerical results of selection upon polyhybrids.. 531

NADLER, J. ERNEST, Effects of temperature on length of vestigial wing in *Drosophila virilis*.................................... 584

ICHIJIMA, K., Cytological and genetic studies on Fragaria........ 590

HEPPNER, MYER J., Further evidence on the factor for bitterness in the sweet almond.. 605

[This number of GENETICS (11: 503-606) was issued April 22, 1927]

Sonderabdruck aus dem „Biologischen Zentralblatt“
47. Band. Heft 2. Februar 1927.

Über genetische Pseudofertilität bei der selbststerilen Nicotiana Sanderae hort.[1]

Von

Friedrich Brieger

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Mit 1 Kurve

Die von Correns (1912) begonnene genetische Analyse der Selbststerilität bei höheren Pflanzen ist durch die Untersuchungen Easts und seiner Mitarbeiter, besonders durch die Arbeit von East u. A. J. Mangelsdorf (1925) wesentlich gefördert worden. Danach wird die Selbststerilität sowie auch die Kreuzungssterilität bei selbststerilen Pflanzen durch eine Serie von multiplen Allelomorphen S_1, S_2, S_3 etc., die einen Einfluß auf die Wachstumsgeschwindigkeit der Pollenschläuche im Griffel ausüben, bedingt. Wenn im Griffel und in einem Pollenschlauch, der durch den Griffel wächst, das gleiche Sterilitätsallelomorph vorhanden ist, dann ist die Wachstumsgeschwindigkeit der Pollenschläuche so gering, daß während der normalen Lebensdauer einer Blüte eine Befruchtung nicht erfolgen kann. Pollenschläuche, die das gleiche Sterilitätsallelomorph enthalten, wachsen jedoch in den Griffeln anderer Pflanzen, die dieses Allelomorph nicht besitzen, mit genügender Geschwindigkeit, um die Befruchtung auszuführen.

Diese Theorie, die zuerst für *Nicotiana Sanderae hort.* (*N. alata* Link u. Otto × *N. Forgetiana hort.*) bewiesen wurde, scheint eine gewisse Allgemeingültigkeit zu besitzen. Unabhängig von East u. A. J. Mangelsdorf (1925) gelangte Filzer (1926) bei der Fortsetzung der Versuche von Lehmann mit der selbststerilen *Veronica syriaca* zu der gleichen Annahme. Die Experimente von Baur (1919) mit dem selbststerilen *Antirrhinum Segovia* Baur stimmen ebenfalls mit den Anforderungen der neuen Theorie überein. Die Kreuzungsresultate der späteren Generationen des selbststerilen *Verbascum phoeniceum* L. in den Versuchen von Sirks (1926) lassen sich unter Zunahme gewisser Hilfshypothesen auf die gleichen Gesetzmäßigkeiten zurückführen. Die abweichenden Resultate der ersten Generationen beruhen vielleicht teilweise darauf, daß mehr als eine Serie von Selbststerilitätsfaktoren vorhanden ist, oder daß modifizierende Faktoren existieren, die die Sterilität abschwächen und durch die von Sirks durchgeführte Selektion eliminiert werden konnten.

Die Versuche von Correns (1912) an Cardamine lassen sich dagegen nicht auf das East-Mangelsdorfsche Schema zurückführen, sondern das ursprüngliche bifaktorielle Schema bleibt hier gültig.

---

[1] Die vorliegende Untersuchung wurde im Bussey Institution, Harvard University, ausgeführt. Dem International Education Board, New York, durch den mir der Aufenthalt in Amerika ermöglicht wurde, sowie vor allem Herrn Professor Dr. E. M. East, in dessen Abteilung die Versuche durchgeführt wurden, möchte ich auch noch an dieser Stelle meinen ergebensten Dank aussprechen.

Während bisher alle auf ihre Sterilitätsverhältnisse geprüften Pflanzen von *Nicotiana Sanderae* die auf Grund der East-Mangelsdorfschen Theorie vorausgesagten Resultate gaben, trat im Sommer 1925 innerhalb einer Population von mehreren tausend Individuen, die von A. J. Mangelsdorf und mir auf ihre Sterilitätsbeziehungen geprüft wurden, eine Pflanze auf, bezeichnet als 46 LE, welche abweichende Resultate gab. Die Abweichungen beschränkten sich lediglich auf den Grad der Sterilität. Bezüglich aller anderen Eigenschaften, wie z. B. Blütenfarbe etc. war die Pflanze vollkommen normal.

Die Pflanze 46 LE stammte aus einer Kreuzung vom Typus: $S_2 S_3 \times S_1 S_3$. Die erhaltene Familie LE bestand neben der abweichenden Pflanze 46 LE aus 59 $S_1 S_3$-Pflanzen und aus 52 $S_1 S_2$-Pflanzen (erwartet je 56). Die reziproke Kreuzung gab nur normale Pflanzen, und zwar 56 $S_1 S_2$-Pflanzen und 50 $S_2 S_3$-Pflanzen (erwartet je 53).

Von ihren Geschwisterpflanzen unterschied sich die Pflanze 46 LE zunächst dadurch, daß sie vollkommen selbstfertil war. Bei der Prüfung mit den drei Sterilitätsallelomorphen, S_1, S_2 und S_3, die in den damaligen Populationen enthalten waren, ergab sich Fertilität mit S_2- und S_3- und Sterilität nur mit S_1-Pollen. Damit war zunächst gezeigt, daß die Pflanze 46 LE den Faktor S_1 enthielt. Die reziproken Prüfungen mit Pollen von 46 LE auf den Testpflanzen bekannter Konstitution konnten leider nicht durchgeführt werden, da die Pflanze vorher abstarb.

Eine kleine Probe von Samen von 46 LE nach Selbsten wurde darauf im Gewächshaus hochgezogen. Von den erhaltenen 22 Pflanzen besaßen 16 normalen Wuchs und 6 den abweichenden Wuchstypus, der für homozygotische $S_3 S_3$-Pflanzen charakteristisch ist (beschrieben und abgebildet von East u. A. J. Mangelsdorf 1926). Diese sechs Pflanzen starben ab, ehe sie zur Blüte kamen, was bei dieser Kümmerform die Regel ist. Es ließ sich also nicht durch Bestäubungen einwandfrei nachprüfen, daß sie die Konstitution $S_3 S_3$ hatten. Von den 16 normalen Pflanzen wurden alle bis auf eine, 15—46 LE, die erkrankte und einging, weitergeprüft. Von ihnen wird im folgenden ausschließlich die Rede sein.

Wie Tabelle 1, Reihe 2 zeigt, waren alle Pflanzen steril auf S_1-S_3-Testern und konnten danach entweder die Konstitution $S_1 S_1$ oder $S_1 S_3$ haben; die Möglichkeit, daß sie $S_3 S_3$-Pflanzen wären, schied aus, da sie nicht den für solche Formen charakteristischen Kümmerwuchs hatten. Da ferner alle Pflanzen fertil auf $S_1 S_1$-Testern waren (Tabelle 1, Reihe 1), schied auch die Konstitutionsformel $S_1 S_1$ aus. D. h. die Pflanzen hatten sämtlich die Konstitution $S_1 S_3$.

Damit ist nun auch die Analyse der Pflanze 46 LE indirekt weiter gebracht. Es war bereits direkt durch die Sterilität mit S_1-Testpollen gezeigt worden, daß sie den Faktor S_1 enthielt, was nun weiterhin durch das Auftreten von S_1 in den Nachkommen bestätigt wird. Da die Nachkommen aber außerdem den S_3-Faktor besaßen, muß 46 LE

Tabelle 1. Die Sterilitätsverhältnisse der S_1S_3-Nachkommen von Pflanze 46 LE.

	auf S_1S_1 ♀	auf S_1S_3 ♀	selbst	mit S_1S_1 ♂	mit S_1S_3 ♂
1—46 LE	+	—	—	—	—
2—46 LE	+	—	—	—	—
3—46 LE	+	—		—	+
4—46 LE	+	—	+	—	+
5—46 LE	+	—	+	+	+
6—46 LE	+	—	+	—	+
7—46 LE	+	—	+	—	+
8—46 LE	+	—		—	+
9—46 LE	+	—	+	+	+
10—46 LE	+	—	+	—	+
11—46 LE	+	—	+	—	+
12—46 LE	+	—	+	—	+
13—46 LE	+	—	—	—	—
14—46 LE	+	—	+	—	+
16—46 LE	+	—	+	—	+

+ Fertilität — Sterilität

auch den Faktor S_3 besessen haben, d. h. die Pflanze 46 LE hatte die Konstitution $S_1 S_3$. Sie unterschied sich aber von den typischen $S_1 S_3$-Pflanzen wesentlich dadurch, daß sie fertil für S_3-Pollen war. Das ist direkt bewiesen durch die Fertilität mit S_3-Testpollen wie auch indirekt durch das Auftreten von $S_3 S_3$-Pflanzen in der Nachkommenschaft nach Selbsten.

Die Pflanze 46 LE war selbstfertil gewesen. Von den daraufhin geprüften Nachkommen waren 10 selbstfertil und drei selbststeril (die Pflanzen 1—46 LE, 2—46 LE und 13—46 LE, Tabelle 1, Reihe 3). Die Selbstfertilität von 46 LE wurde also vererbt, und zwar war diese Pflanze heterozygotisch für den neuen Fertilitätsfaktor „P“, wie er von jetzt an bezeichnet werden soll.

Da die Elternpflanzen von 46 LE, die beiden Pflanzen L1 und 8 L1 Q2, ihre Geschwisterpflanzen in den bereits erwähnten Familien LE und EL sowie die Nachkommen anderer Kreuzungen, in denen die beiden Elternpflanzen verwendet worden waren, nur normal sterile Pflanzen gaben, muß der neue Faktor „P“ von 46 LE einem einmaligen, im übrigen aber seltenen Mutationsvorgange seine Entstehung verdanken. Irgendeine Verunreinigung kann auch nicht vorliegen, da die Pflanze 46 LE homozygotisch rezessiv für den Faktor „c“ war, der Fehlen jeden Anthokyans in der Pflanze bedingt (Brieger und A. J. Mangelsdorf 1926), und der damals nur in streng selbststerilen Sippen vorhanden war.

Der Faktor „P“ in der Mutante 46 LE hatte bewirkt, daß die S_3-Pollenschläuche in ihrem Wachstum im Griffel von 46 LE nicht gehindert waren, wie es in einer $S_1 S_3$-Pflanze an sich zu erwarten wäre, sondern daß nur die S_1-Pollenschläuche von der Befruchtung ausgeschlossen wurden. Wie die Resultate in Tabelle 1, Reihe 4 und 5 zeigen war das Gleiche, Sterilität mit S_1 und Fertilität mit S_3, bei zehn Individuen der S_1S_3-Nachkommenschaft von 46 LE der Fall. Drei der Nachkommen waren vollkommen steril mit S_1 und S_3 und infolgedessen auch selbststeril. Zwei schließlich waren fertil mit S_1; ob sie auch fertil mit S_3 waren, kann erst gesagt werden, wenn ihre Nachkommenschaft analysiert ist, da sie mit S_3S_3-Pollen nicht geprüft werden konnten.

Dieser zunächst etwas kompliziert scheinende Sachverhalt kann durch folgende Arbeitshypothese erklärt werden. Der Faktor „P“ ist gekoppelt mit den Sterilitätsallelomorphen „S“ und seine Wirkung geht dahin, daß er die Sterilitätswirkung des mit ihm gekoppelten Allelomorphs aufhebt, wenn die Pflanze als Weibchen verwendet wird. Als Männchen verhielten sich ja die Pflanzen, die den Faktor „P“ enthielten, trotzdem vollkommen wie normal selbststerile Pflanzen von der Konstitution S_1S_3.

Wenn wir das normale, indifferente Allelomorph zu „P“ mit „p“ bezeichnen, hatte die Mutante 46 LE die Konstitution: S_1p S_3P. Es waren in ihr, wie gezeigt, die S_1-Pollenschläuche von der Befruchtung ausgeschlossen, während die S_3-Pollenschläuche infolge der Anwesenheit der Gruppe S_3P nicht im Wachstum gehemmt waren. Die gleichen Koppelungsbeziehungen mußten sich bei der Mehrzahl der Nachkommen nach Selbsten finden. 10 der 15 geprüften S_1S_3-Nachkommen waren ja auch steril mit S_1 und fertil mit S_3, hatten also die gleiche Konstitution wie die Mutterpflanze, nämlich S_1p S_3P. Einige Nachkommen mußten durch crossing-over entstanden sein. Es konnte entweder in der Gruppe S_3P das „P“ mit „p“ vertauscht worden sein, so daß normal sterile Pflanzen resultieren mußten, die die Konstitution S_1p S_3p besaßen. Drei solche Pflanzen wurden gefunden (die Pflanzen 1—46 LE, 2—46 LE und 13—46 LE). Oder es konnte in der Gruppe S_1p das „p“ mit „P“ ausgetauscht worden sein, wodurch für S_1-Pollen fertile Pflanzen mit der Gruppe S_1P gebildet wurden. Zwei solche Pflanzen wurden gefunden (die Pflanzen 5—46 LE und 9—46 LE). Ob diese beiden Pflanzen die Gruppe S_3P oder S_3p enthielten, konnte nicht festgestellt werden, da der dazu nötige S_3S_3-Pollen nicht zur Verfügung stand.

Die bisher vorliegenden Versuchsergebnisse stimmen mit den Forderungen der Arbeitshypothese überein. Es sind jedoch noch ausgedehntere Versuche notwendig, ehe sie aus dem Stadium einer Arbeitshypothese in das einer wohl fundierten Theorie rückt. Es wäre von allgemeinem theoretischen Interesse, wenn Fälle ganz sicher gestellt würden, in denen die Gene je nach ihren Koppelungsbeziehungen sich phänotypisch verschieden äußern, wo die Koppelungsgruppe, das heißt das Chromosom sich in der phänotypischen Wirkung als eine übergeordnete physiologische Einheit auswirkt, und nicht die Gene selbst diese

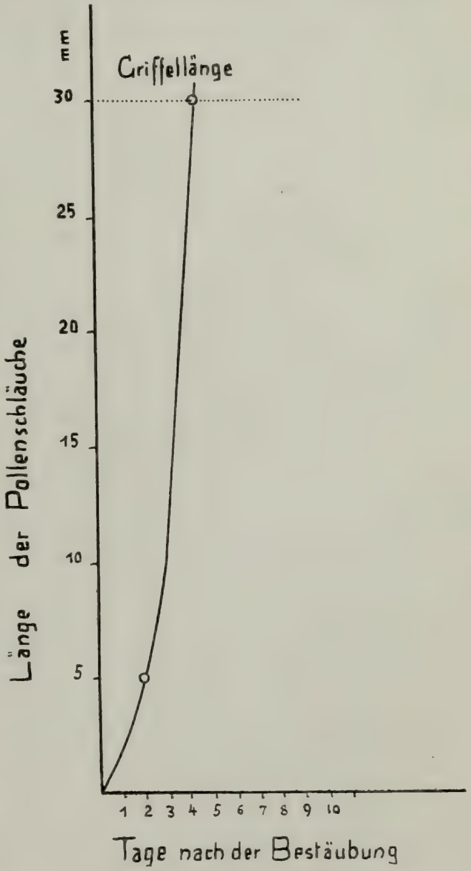
Einheiten sind. Der einzige etwas ähnliche Fall, der mir bekannt geworden ist, ist von Sturtevant bei *Drosophila melanogaster* beschrieben worden (1925). Es kommt hier gelegentlich durch besondere Austauschprozesse vor, daß die beiden allelomorphen Gene für „bar" (bandförmiges Auge) in einem Chromosom dicht nebeneinander zu liegen kommen. Der phänotypische Effekt der beiden Gene ist verschieden, wenn sie zusammen in einem Chromosom liegen, oder wenn sie in je einem der beiden homologen Chromosomen liegen. In unserem Falle handelt es sich jedoch um zwei verschiedene Gene, den Sterilitätsfaktor „S" und das Gen für Pseudofertilität „P", die ganz normale Koppelungsbeziehungen zeigen und, nach den bisher vorliegenden kleinen Zahlen, sogar ziemlich lose gekoppelt sind, also ziemlich weit voneinander entfernt im Chromosom liegen. Das Gemeinsame der beiden Fälle liegt darin, daß es sich anscheinend beide Male um einen „Lageeffekt" handelt, indem die Koppelungsbeziehungen, d. h. die Lage im Chromosom die phänotypische Wirkung bestimmen.

Während die Annahme einer Koppelung zwischen dem neuen Faktor „P" und den Sterilitätsallelomorphen „S" durch die vorliegenden Daten wahrscheinlich gemacht wird, sind die Zahlen zu klein, um irgendwelche begründeten Angaben über den Grad der Koppelung zu machen. Die Koppelungsbeziehungen zwischen „P" und dem grundlegenden Farbfaktor „C", der nach Brieger u. A. J. Mangelsdorf (1926) mit den Sterilitätsallelomorphen gekoppelt ist und etwa 18% crossing-over gibt, müssen auch noch geprüft werden. Die Versuche über diese Punkte sind im Gange.

Die Feststellungen über den Faktor „P" haben auch einigen Wert bei der Analyse der Physiologie der Selbststerilität.

Die Wachstumskurve kompatibler Pollenschläuche entspricht der Kurve einer autokatalytischen Reaktion, wie zu erwarten war und von East u. Park (1918) auch nachgewiesen wurde. Verglichen mit dieser Normalkurve stellen die Kurven inkompatibler Pollenschläuche Hemmungskurven dar. Die Hemmung kann verschieden weit gehen. Es kann so weit kommen, daß das Wachstum gar nicht mehr beschleunigt wird, sondern stetig ist, d. h. daß die Wachstumskurve eine gerade Linie ist. Wie diese Hemmung zustande kommt, kann man sich damit vorstellen, „that easily diffusible substances pass out of the cells of the style and into the membrane of the pollentube" (East 1926, p. 413). Treffen sich dann innerhalb des Pollenschlauches „identische" Substanzen, d. h. Substanzen, die von dem gleichen Sterilitätsallelomorph in Griffel und Pollenschlauch produziert sind, so tritt eine Art Vergiftung des Pollenschlauches ein, der nur noch langsam weiter wächst. Hierbei spielt das Gewebe der weiblichen Pflanze, der Griffel, insofern eine mehr aktive Rolle, als von ihm die diffusiblen Substanzen zunächst gebildet werden.

Die Wirkung des Faktors „P" besteht nun darin, daß die Bildung des Sterilitätsstoffes, der von dem mit ihm gekoppelten Sterilitätsallelomorph produziert werden sollte, in den Griffeln unterbleibt. Die Pflanze verhält sich als Weibchen ganz, wie wenn sie die betreffenden Sterilitätsallelomorphen gar nicht enthielte. Dagegen verhält sich dieselbe Pflanze als Männchen vollkommen normal und gibt die erwarteten Sterilitätsreaktionen. Wenn die Pollenschläuche die Sterilitätsstoffe in einem anderen Griffel vorgebildet finden, reagieren sie darauf, ohne daß jetzt der Faktor „P“ irgendeinen Einfluß hätte.



In Abbildung 1 ist die Wachstumskurve von Pollenschläuchen der Pflanze 16—46 LE, die den Faktor „P“ enthält, nach Selbsten wiedergegeben. Sie zeigt deutlich das für ungehemmte Pollenschläuche charakteristische stark beschleunigte Wachstum, an Stelle der nach Selbsten sonst auftretenden Hemmungskurve.

Insofern als der neue Faktor nicht absolute Fertilität bedingt, sondern nur die Wirkung der Sterilitätsallelomorphen bis zu einem gewissen Grade maskiert, ist er nur ein Faktor für Pseudofertilität, und nicht für Fertilität.

Das Auftreten des Faktors „P“ hat schließlich noch ein gewisses Interesse von phylogenetischen Gesichtspunkten aus. Es handelt sich ja hier um eine einwandfrei nachgewiesene Mutation, durch die ein biologisch sehr wichtiger Prozeß betroffen wird. Die Selbstferilität wird in Selbststertilität umgewandelt. Die „ökologische Bedeutung“ dieser Änderung auszuwerten, ist eine Frage der reinen Spekulation. Daß eine solche Änderung vorkommt, scheint mir an sich schon wichtig.

Ein Überführen der Selbststerilität in Selbstfertilität bei *Nicotiana Sanderae*, bezw. den beiden Elternarten *N. alata* und *N. Forgetiana* ist sonst nur durch Kreuzen mit der dominant selbstfertilen *Nicotiana Langsdorffii*, mit der man vollkommen fertile Bastarde erhält, möglich. Es wird von besonderem Interesse sein, zu prüfen, ob Beziehungen zwischen dem Pseudofertilitätsfaktor „P“ und dem von East (1915) beschriebenen Fertilitätsfaktor „F“ von *Nicotiana Langsdorffii* bestehen.

Zusammenfassung

1. Innerhalb einer Population von mehreren tausend selbststerilen Pflanzen von *Nicotiana Sanderae hort.*, die auf ihre Sterilitätsbeziehungen geprüft wurden, trat eine vollkommen selbstfertile Pflanze, bezeichnet als 46 LE, auf.

2. Die Selbstfertilität war erblich, und zwar spalteten die Nachkommen von 46 LE nach Selbsten annähernd im Verhältnis von 3 selbstfertil : 1 selbststeril.

3. Die Pflanze 46 LE enthielt die beiden Sterilitätsallelomorphen S_1 und S_3. Die Prüfung von 46 LE und ihrer Nachkommen mit S_1- und S_3-Testpollen gab etwas komplizierte Verhältnisse, die durch die folgende Arbeitshypothese erklärt werden können:

4. Der neue Pseudofertilitätsfaktor „P“ ist mit den Sterilitätsallelomorphen „S“ gekoppelt, und seine Wirkung geht dahin, daß er die Wirkung des mit ihm gekoppelten Allelomorphs aufhebt, wenn die betreffende Pflanze als Weibchen benutzt wird.

5. Werden dieselben Pflanzen als Männchen benutzt, so hat der Faktor „P“ keinen Einfluß auf die Sterilitätsverhältnisse.

6. Die Annahme über die Wirkungsweise des Faktors „P“ auf die Wachstumsgeschwindigkeit der Pollenschläuche fügt sich den hier vertretenen Vorstellungen über die Physiologie der Selbststerilität ein.

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INHERITANCE OF TRIMORPHISM IN LYTHRUM SALICARIA

BY

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Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 13, No. 3, pp. 122-124, March, 1927.

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 13, No. 3. March, 1927.

INHERITANCE OF TRIMORPHISM IN LYTHRUM SALICARIA

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Communicated February 18, 1927

Numerous crosses between the three types of flowers found in *Lythrum salicaria* have given adequate basis for the following conclusions:

(1). The self-sterility of the species is not interpretable on the selective pollen-tube growth hypothesis found applicable to Nicotiana, Antirrhinum and Veronica.

(2). The two kinds of pollen in each form carry the same hereditary factors so far as the inheritance of trimorphism is concerned. Illegitimate pollinations with pollen from anthers not at the same level give the same results as legitimate pollinations with pollen from anthers at the same level.

(3). The flower differences are conditioned by three genes. Long-styled flowers are the ultimate recessive. Short-styled flowers are determined by a factor A, and may or may not carry Mid. Mid-styled flowers are conditioned by duplicate factors M_a and M_b in the same linkage group. There is about 10 per cent crossing-over in both the male and the female gametes, though there appears to be slightly less crossing-over in the females than there is in the males. The presence of either or both of the factors conditioning Mid in the homozygous condition produces a lethal effect.

I have never obtained progeny from selfed Shorts, but all three forms have been obtained by other workers. Numbers were too small, however, to give dependable ratios. Longs produce only Longs. Mids presumably of constitution $\frac{M_aM_b}{m_am_b}$, when selfed, yielded $75L$:$194M$. From this ratio it is clear that the cross-over percentage is about 10. The Mids produced will on theory be 57.6 per cent of type $\frac{M_aM_b}{m_am_b}$, 6.4 per cent of each of the two types $\frac{M_am_b}{m_am_b}$ and $\frac{m_aM_b}{m_am_b}$ and 0.71 per cent of the type $\frac{M_am_b}{m_aM_b}$.

A series of Mids of these constitutions, presumably in somewhere near the proportion given above, were crossed with a Mid of constitution $\frac{M_am_b}{m_aM_b}$. The result in 17 families was a ratio of $3L$: $117M$. A similar lot of Mids, though probably not having the same constitutions in the same proportion, were crossed with another Mid of constitution $\frac{M_am_b}{m_aM_b}$. In a series of 31 families the resulting ratio was $15L$: $507M$. There was just under 3 per cent Longs in each case. Theoretically, when Mids of constitution $\frac{M_am_b}{m_aM_b}$ are crossed (1) with Mids of constitution $\frac{M_aM_b}{m_am_b}$ the result should be 4.3 per cent Longs, (2) with Mids of constitution $\frac{M_am_b}{m_am_b}$ or $\frac{m_aM_b}{m_am_b}$ the result should be 3.3 per cent Longs, and (3) with Mids of constitution $\frac{M_am_b}{m_aM_b}$ the results should be 0.5 per cent Longs. A mixture of these four types of crosses might well be expected to give 3 per cent Longs.

When Longs are crossed with Mids there should be two very different ratios. Mids $\frac{M_am_b}{m_am_b}$ and $\frac{m_aM_b}{m_am_b}$ should give 50 per cent Longs; Mids $\frac{M_aM_b}{m_am_b}$ should give 45 per cent Longs. We obtained 53.3 per cent Longs with 491 plants. The other ratio is when a Mid of constitution $\frac{M_am_b}{m_aM_b}$ is crossed with a Long. Here 5 per cent Longs is expected. We obtained 8 per cent Longs in a total of 635 plants.

Longs crossed with Shorts which do not carry Mid should give $1L$:$1S$ We obtained $437L$: $481S$.

Longs crossed with Shorts which carry Mid should also give two ratios that are similar and one that is wholly different from the other two. A $25L$: $25M$: $50S$ ratio is given with Short $\frac{A}{a}\,\frac{M_a m_b}{m_a m_b}$ or $\frac{A}{a}\,\frac{m_a M_b}{m_a m_b}$; a $22.5L$: $27.5M$: $50S$ is given with Short $\frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$. Presumably the second type is the commoner one. In such crosses we obtained $95L$: $115M$: $225S$—a percentage ratio of $21.8L$: $26.4M$: $51.8S$. The other type of Short, $\frac{A}{a}\,\frac{M_a m_b}{m_a M_b}$, when crossed with Long, gives a ratio of $2.5L$:$47.5M$:$50S$. In such crosses we obtained $9L$: $77M$: $116S$—a percentage ratio of $4.4L$: $38.1M$:$57.5S$.

Crosses between Mids other than those of constitution $\frac{M_a m_b}{m_a M_b}$ with Shorts *known not to carry Mid*, give ratios approaching $25L$: $25M$: $50S$. Slight departures decrease the Longs and increase the Mids. In a series of crosses our results were $126L$: $125M$: $243S$.

Crosses between Mids of constitution $\frac{M_a m_b}{m_a M_b}$ with *Shorts known not to carry Mid*, give the very different ratio of $2.5L$:$47.5M$:$50S$. We obtained $5.8L$:$41.9M$:$52.3S$, in a total of 86 plants.

Crosses between Mids and Shorts known to carry Mid can be of various types. We will report only one of our experiments here because of its critical value. Two crosses were made between Mids and Shorts in which the parental plants came from the same families. The crosses were made in such a way that, according to the simple two-factor hypothesis used by earlier writers for the interpretation of trimorphism, they should be duplicates of each other. Yet they gave quite different results. The first cross, made reciprocally, yielded $4L$:$87M$:$134S$, a ratio of $1.8L$: $38.7M$: $59.5S$. Now this is a reasonable fit either for a cross $\frac{a}{a}\,\frac{M_a M_b}{m_a m_b} \times \frac{A}{a}\,\frac{M_a m_b}{m_a M_b}$, or for a cross $\frac{a}{a}\,\frac{M_a m_b}{m_a M_b} \times \frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$, both of which yield $2.2L$: $47.8M$: $50S$. Assuming that we are dealing with a cross of the first type, another Short parent from the same family may be a cross-over Short of constitution $\frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$. Such a Short crossed with the same Mid or a Mid of constitution identical with that used in the first cross should give a ratio of $14.4L$:$35.6M$:$50S$. What we actually obtained was a ratio of $20L$:$54M$:$71S$, which is a percentage ratio of $13.8L$:$37.2M$:$49.0S$.

POLLEN-TUBE GROWTH IN LYTHRUM SALICARIA

BY
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Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 13, No. 4, pp. 253-255, April, 1927.

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 13, No. 4. April, 1927.

POLLEN-TUBE GROWTH IN LYTHRUM SALICARIA

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Communicated March 5, 1927

All investigators who have studied pollen-tube growth in self-sterile plants agree that the immediate cause of the failure to obtain seed after incompatible matings is due to the fact that growth is much slower than it is after compatible matings. The only quantitative investigation of the matter, however, is that of East and Park (1918) on hybrids between *Nicotiana Forgetiana* and *Nicotiana alata*. In this case it was found that pollen-tube growth after an incompatible mating was very nearly linear. The tubes traversed from one-half to two-thirds of the length of the style in about seven days, at which time the flowers usually fell. Acceleration as growth progressed was slight. Growth curves of pollen tubes after compatible matings, on the other hand, showed remarkable acceleration, and fertilization ensued after about three days.

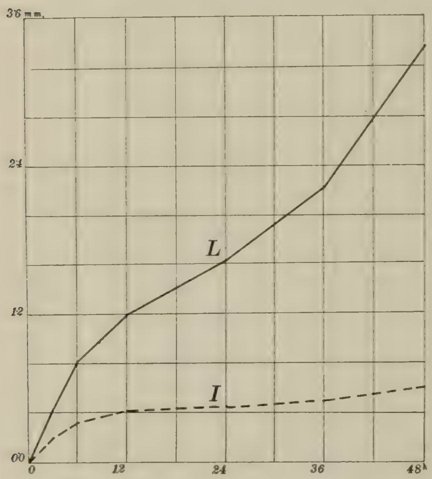

FIGURE 1

Pollen-tube growth in *Lythrum salicaria* after (*L*) legitimate and after (*I*) illegitimate pollination. Mid style × long style.

Since the genetic basis of this self- and cross-incompatibility in Nicotiana is a series of multiple allelomorphs (East and Mangelsdorf, 1925, 1926), and since this interpretation does not appear to fit the phenomenon as exhibited in *Lythrum salicaria*—upon which an extended series of investigations has been carried out in this laboratory—it seemed desirable to determine pollen-tube growth curves for "legitimate" and "illegitimate" unions in this species.

Lythrum salicaria, it will be recalled, is a trimorphic self-sterile species which shows complete fertility only in unions where a given style is pollinated with pollen from stamens of the same tier. Other unions, the "illegitimate" unions, are comparatively infertile, though there is considerable variation among the twelve types of "illegitimate" union.

The material, which consisted of a series of ovaries from a single mid-styled plant pollinated both "legitimately" and "illegitimately" with pollen from a single, long-styled plant, was given to me by Dr. E. M. East. It had been fixed partly in Flemming's medium solution and partly in Bouin's solution and imbedded in paraffin. The styles were fixed separately 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours and 85 hours after pollination. Several styles were available for each stated time.

From this material, longitudinal sections 12μ thick were made, and stained with saffranin, Heidenhain's hämatoxilin or saffranin-gentian violet-orange G. The best preparations were those fixed in Flemming's solution and stained with saffranin. The amount of material investigated is shown in table 1.

It was found that the pollen grains germinated as well after an "illegitimate" as after a "legitimate" pollination, but in the former case the growth was far slower. The average length, the major extremes and the variability of the pollen tubes at the various times after pollination are shown in table 2. The average length of the pollen tubes plotted against time is shown in figure 1. There is a decided difference in the two curves. The curve for a legitimate union appears to be symmetrical with decided ucceleration during later stages. The curve for an illegitimate union is ansymmetrical. Acceleration is shown in the later stages; but it is very slight.

TABLE 1

Styles of *Lythrum salicaria* Investigated

LEGITIMATE UNIONS				ILLEGITIMATE UNIONS			
TIME FIXED AFTER POLLINATION HOURS	NO. OF STYLES INVESTIGATED		TOTAL NO. POLLEN TUBES COUNTED	TIME FIXED AFTER POLLINATION HOURS	NO. OF STYLES INVESTIGATED		TOTAL NO. POLLEN TUBES COUNTED
	FLEMMING	BOUIN			FLEMMING	BOUIN	
3	5	4	188	3	5	4	187
6	5	4	148	6	8	5	239
12	6	5	184	12	6	5	173
24	5	5	173	24	8	4	197
36	6	4	203	36	6	3	193
48	8	4	235	48	6	4	168
60	6	5		60	8	5	
72	2	3		72	2	3	
85	2	3		85	2	3	

TABLE 2

Variability of Pollen-Tube Growth in *Lythrum salicaria*

TIME FIXED AFTER POLLINATION HOURS	MEAN LENGTH MM.	STANDARD DEVIATION	VARIATION AMPLITUDE	TUBE LENGTH IN PER CENT STYLE LENGTH
Leg. 3	0.45	0.35	0.1—1.5	10.0
Leg. 6	0.81	0.45	0.1—1.9	17.3
Leg. 12	1.19	0.60	0.1—3.2	22.7
Leg. 24	1.62	0.70	0.1—3.3	39.8
Leg. 36	2.19	0.95	0.1—3.9	42.8
Leg. 48	3.38	2.20	0.1—7.2	54.5
Leg. 60	The styles begin to fall			
Illeg. 3	0.23	0.07	0.1—0.4	5.1
Illeg. 6	0.34	0.15	0.1—0.7	7.0
Illeg. 12	0.41	0.28	0.1—1.5	8.7
Illeg. 24	0.46	0.35	0.1—2.8	8.5
Illeg. 36	0.48	0.42	0.1—2.9	8.5
Illeg. 48	0.59	0.70	0.1—3.3	10.8
Illeg. 60	The styles begin to fall			

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Origin of the Garden Strawberry

A. J. Mangelsdorf

Reprinted without change of paging, from the Journal of Heredity (Organ of the American Genetic Association), Washington, D. C., Vol. XVIII, No. 4, April, 1927.

ORIGIN OF THE GARDEN STRAWBERRY*

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THE strawberry appears to have been almost completely ignored by the ancients, if one may judge from the failure to mention it in their literature. Sturtevant,[10] who has given us a detailed account of its classical history, found no mention of the fruit in the writings of the early Greeks. Virgil, Ovid and Pliny refer to the plant, but say nothing of its use as an article of diet. More unexpectedly still, Sturtevant discovered no reference to it in the "cook book" of Apacius Coelius, who was supposed to have lived about A. D. 230, nor in the "unusually full and complete treatment of garden, orchard and field products" by Ibnalawam, an author of the tenth century, "nor in *The Forme of Cury,* a roll of ancient English cookery compiled about A. D. 1390 by two master cooks of King Richard II, nor in *Ancient Cookery,* a recipe book of 1381." It is mentioned in several of the sixteenth century herbals, however, and Fletcher[4] quotes Hyll as saying in his "Gardeners' Labyrinth" (1593) that "they be much eaten at all men's tables in the summer time with wine and sugar, and they will grow in gardens until the bigness of the mulberry."

From the scantiness of reference to it, one must conclude that the strawberry received but little attention until the beginning of the seventeenth century, nor is it strange that this should have been the case. Only three species, *Fragaria vesca, F. collina* and *F. elatior,* are indigenous to Europe. All of these species are small-fruited and, though not unpalatable, they lack the flavor and aroma which characterize the American species, *F. virginiana* and *F. chiloensis.*

Introduction of American Species

It is quite likely that the use of the strawberry as a common article of food in the New World antedates its use in the Old. Frezier, who introduced *F. chiloensis* into Europe, found it in fields on the west coast of South America as early as 1712. In a letter to Duchesne[3] (p. 181) he states that: "ils rapportent si abondamment à la ville de la Conception & aux environs, qu'on les vend au marche, comme les autres fruits."

The early New England colonists found the aborigines utilizing the common wild species of that region, *F. virginiana.* Roger Williams, according to Fletcher[4] wrote that the Indians "bruise them in a mortar and mix them with meals and make Strawberry bread." The American species found immediate favor with the early settlers. Fletcher quotes Ralph Hamor as describing "great fields and woods abounding with strawberries, much fairer and more sweete than ours," and an unnamed colonist as writing to his friends in England that the berries were "four times larger and much more exquisitely flavored" than the wild strawberries of England. The size appears to have been somewhat exaggerated, but the comparison as to flavor can hardly be disputed.

F. virginiana was immediately introduced into England where it became widely known under the name of Scarlet or Virginian strawberry. Because of its excellent flavor it rapidly attained popularity and was widely cultivated in gardens. As already mentioned, the first plants of *F. chiloensis* were brought to France from Chile by A. Frezier, a French army officer. According to his account, the ship's supply of water was almost exhausted before the continent was reached, and he was obliged to use a part of his own allowance for the plants. Only five survived. Two of them Frezier gave to Monsieur Roux de Valbonne, nephew

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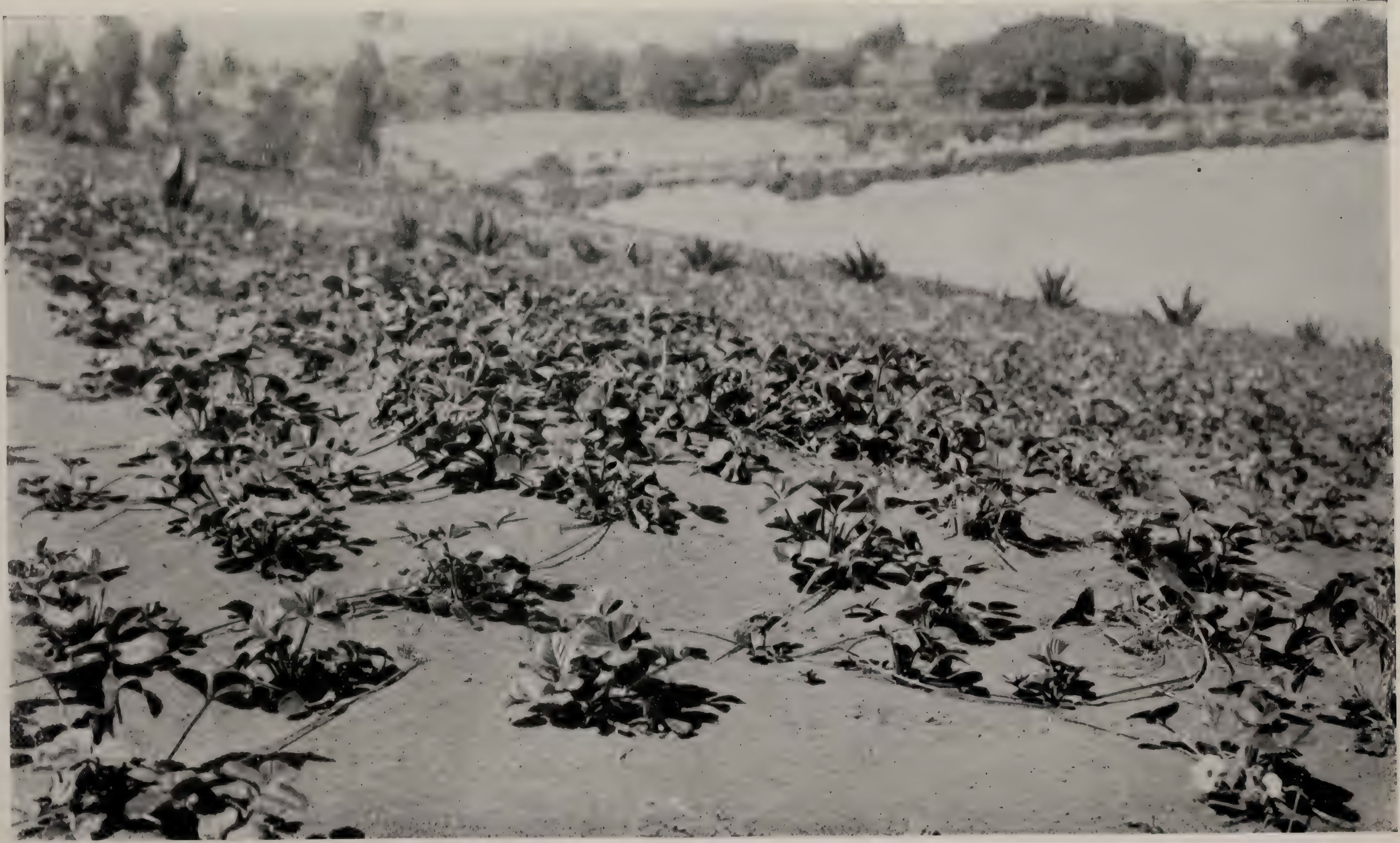
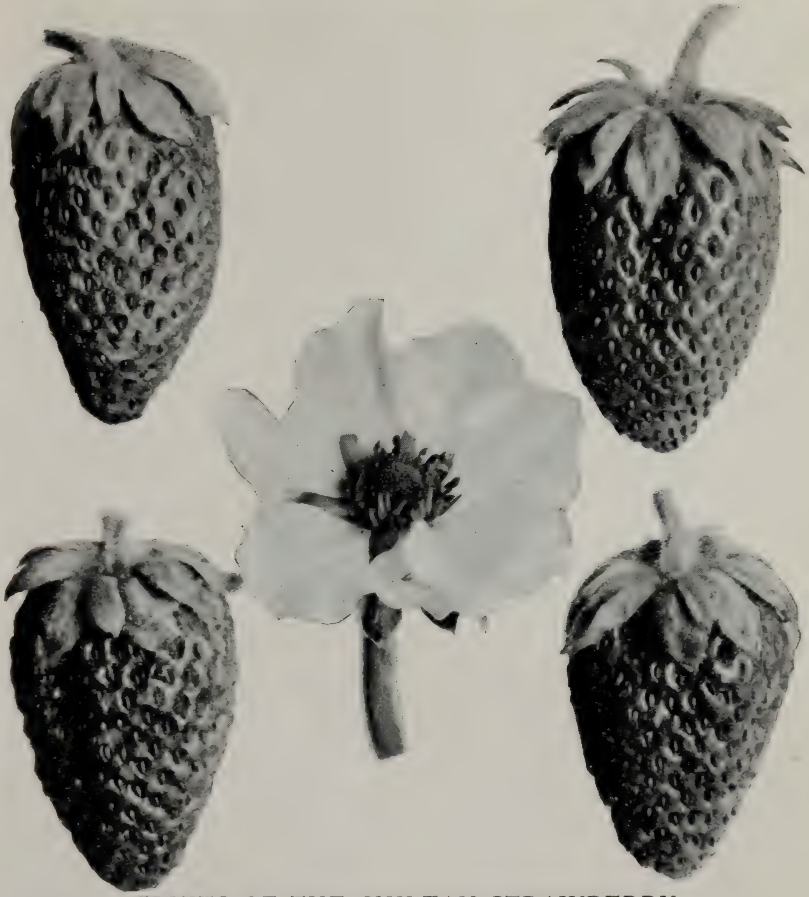
*Contribution from the Laboratory of Plant Genetics, Harvard University, Bussey Institution.



A FIELD OF CHILEAN STRAWBERRIES NEAR AMBATO, ECUADOR

Figure 18

Probably due to the equitable climate *F. chiloensis* bears all the year in this region, with heavier crops of berries in February, August, and December. The species was introduced into Ecuador some time after the Conquest,and today the strawberry plantations in the region of Ambato are the largest in South America. The ground is a very sandy volcanic loam, and the plants are not irrigated. Photograph by Wilson Popenoe, courtesy of Office of Foreign Seed and Plant Introduction, U. S. Department of Agriculture.



FRUITS OF THE CHILEAN STRAWBERRY

Figure 19

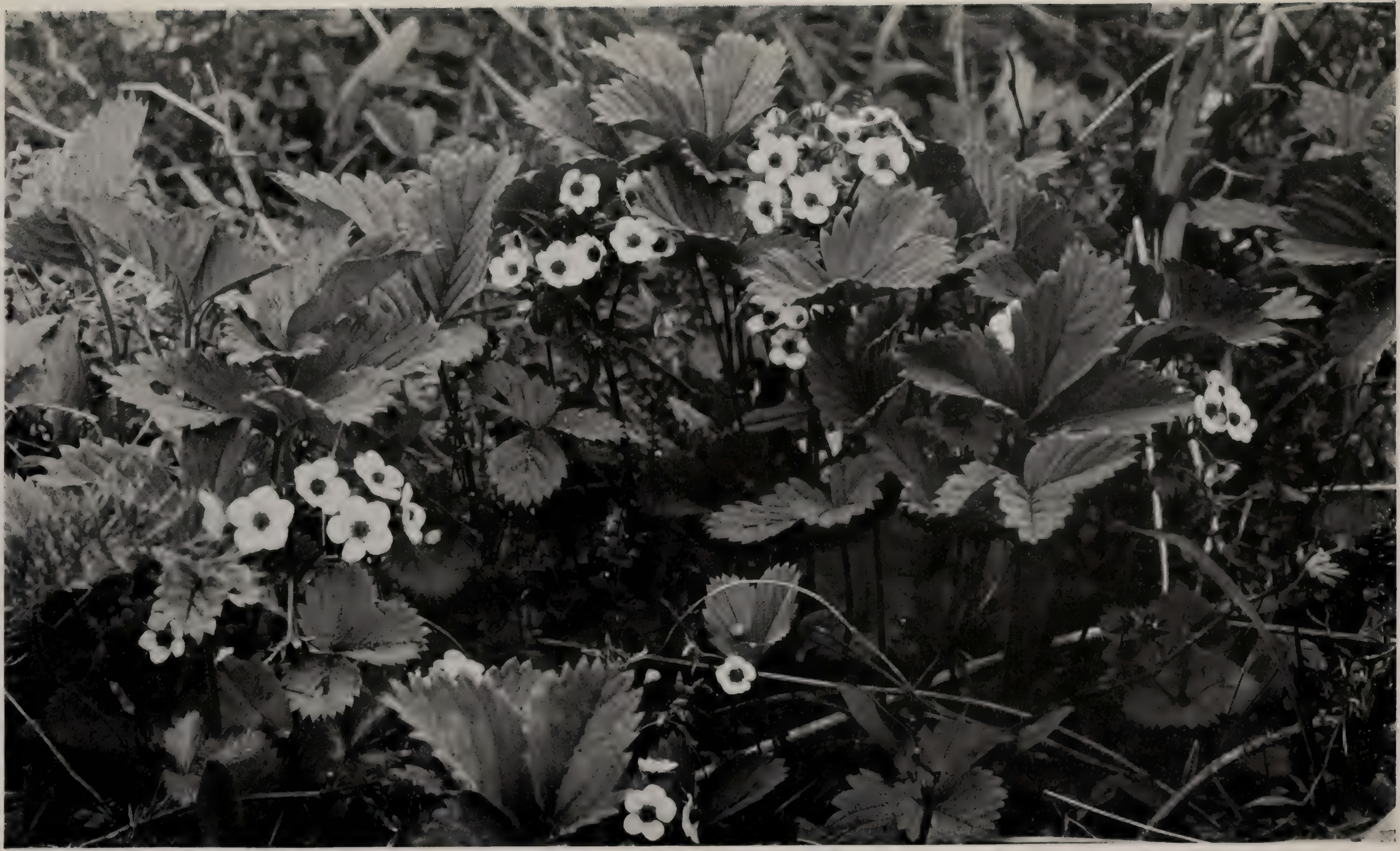
The Chilean strawberry contributed two important characteristics to the garden type—size, and firmness of flesh. None of the garden varieties equal the chilean in the latter characteristic, as in Ecuador the fruits are handled more as we handle apples, being gathered in large boxes and shipped considerable distances by mule and rail without crushing the fruits. Photograph by Wilson Popenoe, courtesy Office of Foreign Seed and Plant Introduction.

of the brothers Bruny, the owners of the vessel, one he gave to M. Antoine Jussieu to be planted in the Royal Garden, another to M. le Pelletier de Souzy, minister of fortifications, and one he retained for himself.

The plants apparently prospered in their new environment and the species was soon disseminated throughout Europe. It met with little enthusiasm, however, since it did not preserve its native fruitfulness in Europe. The reason for this anomaly was discovered by Duchesne: all the plants were pistillate and incapable of self-fertilization. When grown together with *F. virginiana* and *F. elatior* the plants bore well, but presumably this fact was not generally known.

The Large-Fruited Type

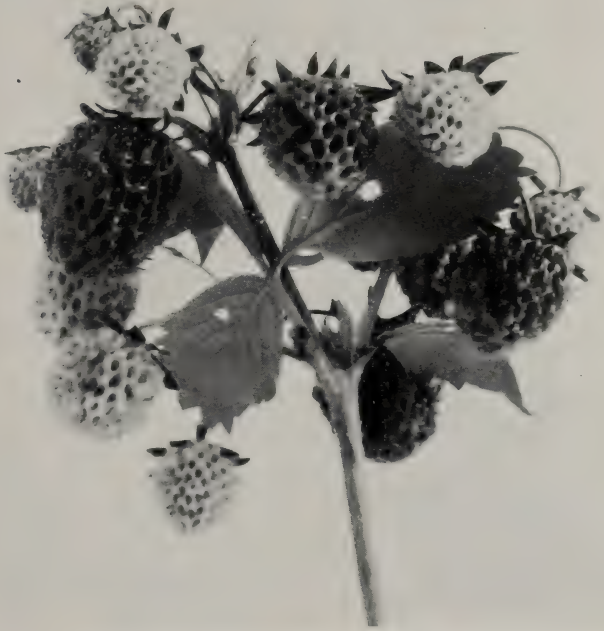
It was not until the middle of the eighteenth century, a number of years after the introduction of *F. chiloensis* into Europe, that the large fruited garden type first appeared. It was designated *F. grandiflora* by Ehrhardt,—a name which we may use as a matter of



THE VIRGINIA STRAWBERRY

Figure 20

Plants of *F. Virginiana* growing wild near Washington, D. C. The Virginia Strawberry was enthusiastically spoken of by the early colonists, and it was very soon introduced into Europe, where its superiority to the European species rapidly gained it a wide distribution. Photograph courtesy of George M. Darrow, U. S. Department of Agriculture.



FRUITS OF VIRGINIA STRAWBERRY

Figure 21

The large fruited garden varieties of strawberries are believed to have arisen from crosses between the two American species, the Chile and Virginia strawberries. This *grandiflora* type first made its appearance in France about 1750, and it is noteworthy that two American species should have crossed the Atlantic to produce the fruit about which a famous divine made the remark: "doubtless God could make a better fruit than a strawberry but doubtless God never had."

convenience. The nature of its origin was unknown, but various explanations were advanced: one that it had been brought from Surinam (where Fragaria is not known to be indigenous), another that it was imported from Carolina. Duchesne, with remarkable acuteness, suspected that it had originated from a cross between *F. chiloensis* and *F. virginiana,* possibly in the vicinity of Brest, where both species had been under cultivation for some time.

The evidence which has accumulated since Duchesne's time points to the correctness of his theory. The *grandiflora* group, as we know it today, displays a wide range of variation, but in all characters except size and robustness it is more or less intermediate between *F. chiloensis* and *F. virginiana.* These forms cross readily with each other and produce fertile hybrids, and both Longley[6] and Ichijima[5a] find that each has 28 pairs of chromosomes. All garden varieties thus far investigated have been ~~found to possess 28 pairs~~ of chromosomes also.

The conclusions of Duchesne as to the origin of the garden type have been quite generally accepted. A few workers, however, have held other opinions. Bailey[1] believes it to be pure *F. chiloensis* modified by culture and by a new environment. He bases his conclusions on observations which he made on plants of *F. chiloensis* which he obtained from Oregon and which, after several years, showed marked changes in their habit of growth. He summarizes his arguments in the following words:

> There is only one conclusion, therefore, which fully satisfies all the demands of history, philosophy and botanical evidence, and this is that the garden strawberries are a direct modification of the Chile strawberry. The initial variation occurred when species were thought to be more or less immutable, and, lacking exact historical evidence of introduction from a foreign country, hybridization was the most natural explanation of the appearance of the strange type. This modified type has driven from cultivation the Virginian berries, which were earlier introduced into gardens; and the original type of the Chilean strawberry is little known, as it tends quickly to disappear through variation when impressed into cultivation. The strawberry is an instance of the evolution of a type of plant, in less than fifty years, which is so distinct from all others that three species have been erected upon it, which was uniformly kept distinct from other species by the botanists who have occasion to know it best, and which appears to have been rarely specifically associated with the species from which it sprung.

Bailey's theory has not been generally accepted. The presence of virginiana-like characters in the garden type is too obvious to be disregarded. Furthermore, it fails to give due consideration to the circumstances under which the *grandiflora* type appeared. There can be no doubt that this type originated in Europe, and it is unanimously agreed that it shows *chiloensis* heritage. Duchesne's account, however, indicates that all the existing *chiloensis* plants in Europe at the time were pistillates, and incapable of self-fertilization. If *grandiflora* came from *chiloensis* parentage, it must necessarily have been through crossing. It is known to have been grown in gardens together with *vesca, elatior* and *virginiana*. The first two have been found by us to give sterile hybrids with *chiloensis,* while the latter gives hybrids which are vigorous and fertile, and which approximate quite closely the *grandiflora* type.

Sturtevant[10] believed *F. vesca* and *F. elatior,* as well as *F. virginiana* and *F. chiloensis,* played a part in the development of the garden type. He states his views as follows:

> The modern varieties under American culture usually have large berries with more or less sunken seeds, with the trusses lower than the leaves, and seem to belong mostly to the species represented in nature by *F. virginiana,* although there are supposed hybridizations with *F. chiloensis* and, in the higher flavored class, with *F. elatior.* Certain it is that in growing seedlings from our improved varieties reversions often occur to varieties referable to the Hautbois and Chilian sorts, from which hybridization can be inferred. I have noted as of common occurrence that seedlings from high-flavored varieties are very likely to furnish some plants of the Hautbois (*F. elatior*) class, and even scarcely, if at all, distinguishable from named varieties of the Hautbois with which there has been opportunity for close comparison. From large berried varieties of diminished flavor, and which occasionally throw hollowed berries, the reversion ocassionally produces plants unmistakably of the Chilian type. In other cases we have noticed reversions to forms of *F. vesca.* These circumstances all lead towards establishing the mingled parentage of our varieties under cultivation, and render the classification of cultivated varieties somewhat difficult.

A similar view is expressed by Richardson[7] when he says:

> Something of the origin of the modern Garden Strawberry is known, but its whole history is not. It springs from an old form of Garden Strawberry, the results of crosses between *vescas,* Alpines and Hautbois. This older form was again crossed with *F. virginiana,* introduced in 1629, and *F. chiloensis,* introduced to Marseilles in 1712 and to England in 1727 by Philip Miller.

Cytological and Genetic Evidence

That *F. vesca* and *F. elatior* have played a part in the development of the garden type appears extremely doubtful in the light of their chromosome numbers and their breeding behavior. *F. vesca* has 7 pairs of chromosomes, and *F. elatior* has 21 pairs, while all of the varieties of the garden type thus far investigated have 28 pairs, as has been stated. Both *F. vesca* and *F. elatior* when crossed with the 28-chromosome forms give hybrids which are almost completely sterile. It must be mentioned that after several years of observation Richardson altered his views as to the participation of *F. vesca* in the parentage of the garden type. He says[8]: "From crosses made with *vesca* × *Daltoniana,* garden varieties and *chiloensis,* I have never obtained free fruiting plants, and am now very strongly inclined to believe that *vesca* has nothing to do with the present garden varieties."

Robbins[9] in his recent text, "The Botany of Crop Plants," says of *F. vesca*: "This strawberry is a native of Europe, but naturalized in the Eastern and Middle States. It has given us our Perpetual and Everbearing varieties," This is incorrect—the everbearing varieties in cultivation at the present time are all of the *grandiflora* type. *F. vesca* is often described as the "monthly" or "everblooming" strawberry. It is probably from this fact that Robbins drew his conclusion as to the origin of our present everbearing varieties.

One point still to be considered is the change which has taken place in the garden type during the past fifty years with reference to sex expression. In *grandiflora,* as in its parents, *chiloensis* and *virginiana,* there is a marked tendency toward diœcism, the pistillate form being in general much more fruitful than the hermaphroditic form. As Darrow[2] has pointed out, the better varieties grown in this country 75 years ago were pistillates; the hermaphroditic varieties were much less fruitful and were grown principally to provide pollen for the more fruitful pistillate varieties. At the present time, however, the most widely cultivated varieties are hermaphrodites, and they are apparently quite as fruitful as any of the pistillates.

A possible explanation of this situation lies in the inheritance of sex expression in the strawberry. The pistillate form is dominant to the hermaphroditic form; and there is some evidence that pistil fertility is conditioned by a number of factors closely linked to the dominant stamen-inhibiting factor. If these assumptions are correct, then these fruitful hermaphrodites have resulted from a selection of crossover types which carry the factors for pistil fertility as well as those necessary for stamen development.

The evidence, then, as to the derivation of the *grandiflora* or garden type may be briefly summarized as follows: It first appeared in Europe in a region where the pistillate form of *F. chiloensis* and the hermaphroditic form of *F. virginiana* had been grown together in gardens for a number of years. It has the same chromosome number as these species and is fully fertile with them. It differs in chromosome number from *F. vesca* and *F. elatior,* and crosses with these species gives sterile hybrids. The evidence is overwhelmingly in favor of the hypothesis advanced by Duchesne in 1766 that it comes from a cross between *F. chiloensis* and *F. virginiana.*

The better varieties of the present day represent selections in which are combined the factors for stamen development and for pistil fertility, together with a high degree of vigor and robustness, resulting presumably from heterosis and from an accumulation, through selection, of favorable growth factors.

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STUDIES ON THE GENETICS OF *FRAGARIA*

A. J. MANGELSDORF AND E. M. EAST
Harvard University, Bussey Institution, Forest Hills, Boston, Massachusetts

Reprinted from GENETICS **12**: 307-339, July, 1927

GENETICS

A Periodical Record of Investigations Bearing on
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Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879.

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Received September 29, 1926

TABLE OF CONTENTS

PAGE
INTRODUCTION 307
Description of species and varieties used 308
  The 7-chromosome group 308
  The 21-chromosome group 310
  The 28-chromosome group 310
  Species from other genera used 311
Technique used in making crosses 311
Description of crosses 313
  Species of the 7-chromosome group crossed *inter se* 313
  7-chromosome species × 21-chromosome species 314
  7-chromosome species × 28-chromosome species 315
  28-chromosome group × 21-chromosome group 320
  Generic crosses 321
  28-chromosome species crossed *inter se* 322
Parthenocarpy, parthenogenesis and apogamy 322
Inheritance of flower color and fruit color in *F. vesca* 325
Millardet's "faux hybrides" 329
CONCLUSIONS 337
LITERATURE CITED 338

INTRODUCTION

In 1894, A. MILLARDET, Professor of Botany at Bordeaux, published a rather extraordinary paper entitled "Note sur l'hybridation sans croisement ou fausse hybridation." In this report the author records the data obtained in a study of Fragaria crosses extending over a period of eleven years, which led him to the conclusion that species hybrids in this genus are not to be regarded as combinations of the two parental types, as in other forms, but that the characters either of the maternal or of the paternal parent are normally reproduced intact in the first and in all succeeding generations. MILLARDET'S unusual results led Count SOLMS-LAUBACH, Professor of Botany at Strassburg, to undertake a further investigation of the genus. His results were conflicting, but he believed he was able to verify, in one cross at least, the occurrence of "hybridation sans croisement" as reported by MILLARDET.

As a result of these investigations the genus Fragaria has frequently been referred to in genetic literature as an exception to the usual rules of inheritance. Since anomalous phenomena are a great stimulus to scientific progress, it seemed desirable to re-examine in detail the behavior of species crosses in this genus. Questions concerning the taxonomic relationship of the various species, the origin of the cultivated varieties and the expression and inheritance of sex gave to the material an added interest.

DESCRIPTION OF SPECIES AND VARIETIES USED

The following species and varieties of Fragaria have been used in these studies. With the exception of a few garden varieties sent to us by commercial firms, they were obtained from the UNITED STATES DEPARTMENT of AGRICULTURE through the kindness of G. M. DARROW and C. V. PIPER.

The chromosome counts were made in this laboratory by Mr. K. ICHIJIMA (1926).

The 7-chromosome group

F. vesca L. Wood strawberry. Fraisier des bois. Rootstock and runners rather slender. Petioles slender, with soft, spreading white hairs; leaflets almost sessile, light green; margin toothed along upper two-thirds, entire along lower third; scapes strong and erect, many flowered, usually overtopping the leaves; flowers small, hermaphroditic, petals concave; calyx lobes spreading or reflexed; stamens hardly as tall as receptacles; styles very numerous, short and relatively thick; fruit roundish or conical, detaching easily, sweet but lacking in the acid flavor characteristic of *F. virginiana;* flesh soft and pulpy or mealy; achenes set superficially.

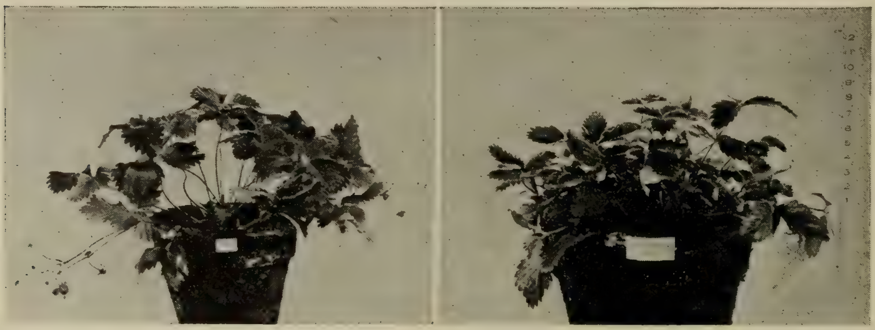

FIGURE 1.—7-chromosome species. At left, *F. vesca* (European); at right, *F. bracteata* (American).

This species has an exceedingly wide range. It is the common wild strawberry of Europe. Forms indistinguishable from it have been reported from Ecuador and Peru, though they may have been introduced, from both Western and Eastern North America, and from Northern Asia.

F. vesca L. From Ecuador, indistinguishable from European form except in shape of berry, which is almost spherical.

F. mexicana Schlecht. Obtained by the UNITED STATES DEPARTMENT of AGRICULTURE from the BOTANICAL GARDEN of BOHEMIA. It appears to be an alpine form of *F. vesca*, presumably introduced into Mexico, and escaped from cultivation. It is difficult to distinguish from *F. vesca;* very free blooming, berries long and conical.

F. vesca Rostrup. A pink-flowered *vesca*.

F. americana alba (Porter). The appearance of the plant is very similar to that of *F. vesca*, though the petioles are somewhat shorter, scapes shorter and weaker. The fruit is white or creamy and the seeds straw-colored.

F. bracteata Heller. Similar to *F. vesca* but the leaves are smaller and the petioles shorter. The growth habit is almost prostrate; petioles and peduncles densely pubescent; scape weak and few flowered; styles short, recurved and few in number; stamens long, overtopping receptacle; fruit spherical, soft and pulpy; achenes slightly sunken in shallow pits. *F. bracteata* ranges from New Mexico to Idaho and Washington.

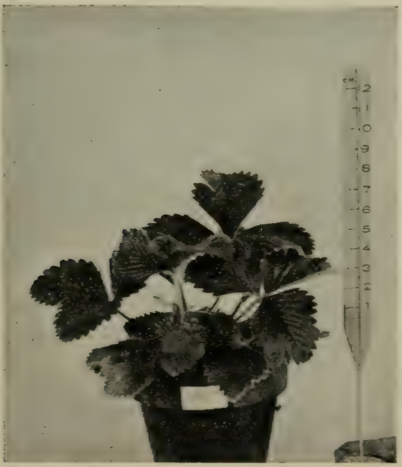

FIGURE 2.—21-chromosome species, *F. elatior*.

F. californica Cham. & Schlecht. California wood strawberry. The growth habit is scarcely distinguishable from that of *F. vesca;* petals very large, thin and finely veined; pistils short, recurved and few in number; stamens long, towering above receptacle; the fruit small, spherical; pulp soft and juicy; achenes slightly sunken in shallow pits. Similar to *bracteata* except in growth habit. Pacific Coast.

The 21-chromosome group

F. elatior Ehr. (*F. moschata* Duchesne). Hautbois strawberry. Similar to *F. vesca* in appearance but leaves, petioles and peduncles heavily pubescent; leaves strongly veined and pale green; flowers large, usually diœcious; styles rather short and thick; petals thick waxy; calyx reflexed; fruit long conical, light red, sweet and with a slightly musky flavor. The range of *F. elatior* includes Central Europe, Sweden, Finland and parts of Russia. Two hermaphroditic garden varieties of this species, Monstreuse Hautbois and Black Hautbois, have been used in these investigations.

The 28-chromosome group

F. virginiana Duchesne. Scarlet or Virginia Strawberry. Rootstock moderately stout; runners long and rather stout; petioles intermediate between *F. vesca* and *F. chiloensis;* leaflets usually stalked, firm in texture and dark green in color; scape about as long as petioles, many flowered, in some forms strong and erect, in others weak and prostrate; usually diœcious; stamens on male flowers long and numerous; calyx often fails to open completely, remaining half closed after petals fall; fruit spherical to short conical, usually with a smooth neck, aromatic and highly flavored; color a bright scarlet; flesh moderately firm; achenes sunk in deep pits. Found in a wide variety of types throughout Eastern North America.

F. virginiana No. 27. An erect, robust, free-flowering pistillate form.

F. glauca Watson. A form of *F. virginiana* from Canada. Scapes strong and many flowered; pubescence scanty throughout.

F. chiloensis L. Chilean strawberry. Rootstock stout, runners strong; petioles stout, densely hairy in some forms, almost glabrous in others; leaflets more or less stalked, thick, leathery and more or less glossy dark green above; pale and quite villous and strongly veined underneath; leaflets wide in proportion to length; outer third crenate with short, rounded teeth, terminal one small; scape usually shorter than leaves, weak, usually few flowered; shape of petals variable in different races; calyx closing after petals fall; usually diœcious; styles rather short; stamens in male flowers long and numerous; fruit pale pink to dull red, more or less translucent, almost spherical, very aromatic; flesh firm; achenes superficial or sunk in shallow pits. This species includes many types which grade into one another. Its range extends from Patagonia to Alaska. A pistillate clone received under the name Oregon No. 2 has been used most extensively in crosses. It agrees with the descriptions of *F. cuneifolia*, a subspecies of *F. chiloensis*.

F. grandiflora Ehr. Common garden strawberry. Believed to have originated from crosses between *F. virginiana* and *F. chiloensis.* Extremely variable, some forms leaning toward *virginiana,* some toward *chiloensis.* In most characters more or less intermediate, but in size of plant, flower and fruit surpassing that of either in our specimens. Flowers often with supernumerary petals. A number of cultivated varieties have been kept under observation.

Species from other genera used

In addition to the Fragaria species enumerated the following two forms have been used in crosses:

Duchesnea indica Andr. Mock or Indian strawberry. Petioles low, spreading; leaves trifoliate, dark green; runners bearing leaves and one-flowered peduncles; petals yellow; bractlets large and dentate at tips; fruit spherical, bright red, watery and insipid; achenes very prominent. Introduced from Northern India. Chromosome counts by ICHIJIMA show 42 chromosomes.

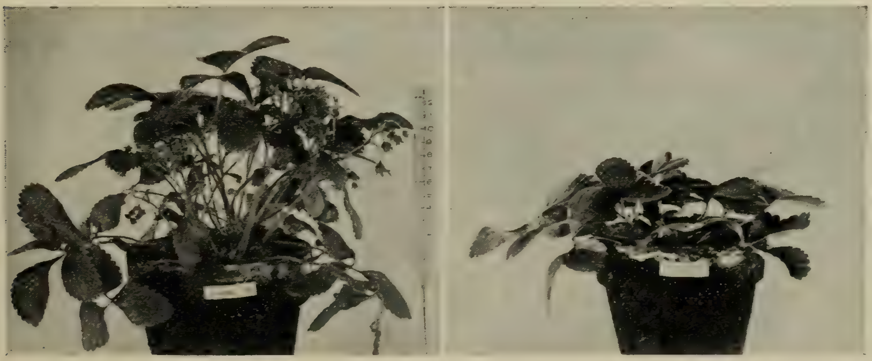

FIGURE 3.—28-chromosome species. At left, *F. virginiana;* at right, *F. chiloensis.*

Potentilla nepalensis hybrid. Horticultural variety Miss Willmott. Plant erect, branching; leaves 5-foliate clothed with long, soft spreading hairs; petals, stamens and pistils rose colored to dark red; pollen bright yellow; receptacle dry and hairy when mature; achenes small. Introduced from the Himalayas. Chromosome number undetermined.

TECHNIQUE USED IN MAKING CROSSES

Emasculation, though tedious because of the large number of anthers, is not difficult. A pair of straight dissecting forceps, the tips of which had been ground down to a fine point, have proved to be best suited to the purpose. At the beginning of the investigation the buds were emasculated immediately before opening. At this stage the work can be done quite rapidly and with a minimum of injury to the receptacle. But though dehiscence does not occur until several hours after the flower has opened, there appears to be some danger when emasculation is done at this stage of functional pollen grains being expressed from the anthers when they are grasped by the forceps, and recently the practice of emasculating two to four days before opening has been followed. This age represents a compromise between opposing considerations; a younger age might be still safer, but the percentage of failure due to the injurious effect of exposing the young pistils is extremely high.

All open flowers and all buds except those to be used, are removed before emasculation and the plant is subjected to a thorough spraying under a stiff stream of water to remove any pollen which may be adhering to the leaves.

Bagging the individual flowers after emasculation proved unsatisfactory, conditions within the paraffin bag apparently being unfavorable to development. Accordingly the plan of removing the emasculated plants to a house containing no other Fragaria individuals was adopted. The plants are set upon inverted saucers placed in the center of a pan of water to isolate them from ants, which visit the flowers diligently. The ventilators are covered with mosquito netting, and flower-visiting insects seldom find their way into the greenhouses, but the plants are covered with wire cages as an additional precaution. Whenever possible the crossing is so planned that only a single kind of pollen is used at a given time. When crosses involving several kinds of pollen are made simultaneously, the plants are removed individually to a second isolation house for pollination in order to guard against pollen being wafted to plants destined for crossing with another species.

The earlier crosses were made without using all of these various precautions, which were adopted only as the extreme difficulty of avoiding contamination became more and more evident. The pollen grains are extremely minute (about 15–20 micra in diameter) and apparently they are readily picked up and carried about by air currents. The extent of the danger of contamination by ants has been recognized only recently. It appears that these insects travel considerable distances from one plant to another, their objective being always the open flowers from which they obtain nectar.

The seeds apparently require an after-ripening period of several months. If sown immediately after they have completed their development, germination is irregular and often is considerably delayed. Scarifying the seeds by rubbing them over emery cloth or fine sandpaper appears to hasten germination, presumably by facilitating the absorption of moisture. Sterilized soil is used in the seedpans, this as a safeguard both against "damping-off" and against the danger of introducing foreign Fragaria seed with the soil.

There is considerable variability in the length of time required by the different species to reach maturity. The 7-chromosome group matures most rapidly, occasional individuals having bloomed at the age of five to six months. The 28-chromosome group requires a longer time, usually from seven to ten months. The 21-chromosome species, *F. elatior*, is the slowest to develop. It seldom blooms before the end of the first year. In all the species backward individuals are found in which blooming may be delayed for several years.

Sowings may be made at any season. Those made in the spring and summer months succeed somewhat better than do those made during the fall and winter, but the difference is not great.

DESCRIPTION OF CROSSES

Species of the 7-chromosome group crossed inter se

The species belonging to this group are all similar to each other morphologically, as well as identical in their chromosome numbers, so it was to be expected that they would cross readily with each other and would give fertile hybrids. Since the expected does not always happen in genetical work, however, the crosses listed below were made for the purpose of determining the facts. In all of the crosses listed, the female parent is mentioned first.

F. californica × *F. bracteata.* F_1 plants vigorous. Ten plants were grown. The three which have thus far bloomed have proved to be fully fertile.

F. californica × *F. vesca rosea.* F_1 plants normal and fairly vigorous. Ten F_1 plants were grown. The two which have bloomed have proved to be pink-flowered, as expected, and were fully fertile.

F. mexicana × *F. americana alba.* Ten F_1 plants were grown. All were normal and moderately vigorous. The seven which have bloomed were red-fruited and fully fertile.

F. americana alba × *F. mexicana.* Ten F_1 plants were grown. All were quite normal and vigorous. The six which have bloomed were fully fertile and were red-fruited.

F. vesca rosea × *americana alba.* Ten F_1 plants were grown. They were exceptionally vigorous. The seven which bloomed were fully fertile and were red-fruited and pink-flowered.

F. vesca rosea×*F. mexicana*. Ten F_1 plants were grown. All were vigorous. The six which bloomed were pink-flowered and fully fertile.

F. vesca (Ecuador)×*F. vesca rosea*. Ten plants were grown. All were vigorous and fully fertile. The pink flower of the *rosea* parent was known to be dominant to white flower. It was expected, therefore, that all of the F_1 plants would prove to be pink-flowered; three, however, were white-flowered. This result could have come about in one of several possible ways; namely, accidental selfing, heterozygosity of the pink-flowered parent, or pseudogamy.[1] The identical *rosea* parent was not retained and it is not known which of these causes was responsible for the appearance of the white-flowered individuals.

F. vesca (Ecuador)×*F. americana alba*. Ten F_1 plants were grown. All were vigorous. The three which have flowered have proved to be red-fruited and fully fertile.

F. vesca (Tiflis, Russia)×*F. vesca rosea*. Ten F_1 plants were grown. All were vigorous. The seven which have flowered were red-fruited and fully fertile.

F. bracteata×*F. vesca rosea*. Ten F_1 plants were grown. They were only moderately vigorous. Six were pink-flowered and four white-flowered. In this case it is fairly certain that the *rosea* parent was heterozygous for pink flower, since the white-flowered plants were intermediate between the two parents in growth habit.

F. bracteata×*americana alba*. Ten F_1 plants were grown. They were vigorous and intermediate between the two parents in growth habit. All bloomed, and proved to be red-fruited and fully fertile.

As was to have been expected from the identity of their chromosome numbers and from their morphological similarities, all of the 7-chromosome species were found to cross readily with each other and to give vigorous fertile progenies. Where discernible differences existed between the two parents the F_1 plants were intermediate, except in flower color and fruit color, pink flower color being dominant to white and red fruit color to white.

7-chromosome species×21-chromosome species

F. vesca×*F. elatior* (Black Hautbois). A number of crosses were made and all set fruit quite readily. The resulting seeds were apparently plump and well developed. Over 400 were sown; of these only three germinated. The seedlings were extremely weak, failed to develop a normal green color in the cotyledons, and died within one to two weeks after germinating without having developed a single leaf. A second sowing of 200 seeds resulted in only 1 seedling which also perished at the age of about two days.

---

[1] Pseudogamy is here used to mean the production of seeds by apogamy or parthenogenesis as a result of the stimulus of pollination.

This result was unexpected because *F. vesca* and *F. elatior* resemble each other fairly closely in general appearance, and especially in the conformation of the fruit. It is all the more surprising, because crosses between the 7-chromosome and the 28-chromosome groups, which differ quite profoundly from each other in morphological characters in addition to the greater discrepancy of their chromosome numbers, give viable F_1 plants.

It is impossible with our present meager knowledge to hazard even a guess as to the reasons for the extreme incompatibility between these two forms. FOCKE (1881) cites KNIGHT as authority for the statement that WILLIAMS succeeded in producing a hybrid from the reciprocal cross, *F. elatior*×*F. vesca.* Our attempts at making the cross in this direction have resulted without exception in failure, not a single flower setting fruit.

7-chromosome species×28-chromosome species

F. vesca×*F. chiloensis.* Flowers of *F. vesca* pollinated with *F. chiloensis* pollen set fruit freely, but a large percentage of the resulting seeds is small and poorly developed. Even the plump seeds germinate poorly, a total of over 1200 fairly well developed seeds producing only 6 seedlings. By the time the latter were a month old it began to be apparent that the uniformity usually obtained in the F_1 generation of species crosses did not exist here. Four of the plants were extremely weak, and produced miniature leaves of a yellowish green color, while two were quite vigorous. As the plants grew older the discrepancy between them increased. The weak individuals failed to improve, their new leaves showing but little increase in size and no improvement in color. The two vigorous plants grew rapidly and in the course of six months attained a size equal to that of their parents. The dark bluish-green color and the thick texture of the *chiloensis* leaf is dominant over the thin, light green character of the *vesca* leaf. The plants resemble the *chiloensis* parent much more closely than *vesca,* although their relatively upright growth habit suggests the *vesca* parent. Neither plant has bloomed. Two of the dwarfs have died, the two which remain showing but little growth. Their leaflets are scarcely more than a centimeter long, while those of their normal sisters are 4–5 centimeters long. Their abnormal color and their minuteness make it difficult to see any resemblance to either of the parents. Possibly they are haploids, but their poorly developed root system has made it impossible to get chromosome counts.

F. americana alba×*F. glauca.* Flowers of *F. americana alba* set fruit quite readily when pollinated by *F. glauca*, but a large proportion of the achenes remain undeveloped. Even those which appear to be normal, germinate poorly. Only one plant was obtained from a sowing of 66 seeds. It is conspicuously lacking in vigor. Its leaves are extremely small, about one-fifth to one-tenth the size of those of the parents. Their relatively thick texture suggests the influence of *F. glauca.* In shape and general appearance they display no particular resemblance to either parent. The plant has never produced runners, nor has it bloomed, although it is now over two years old.

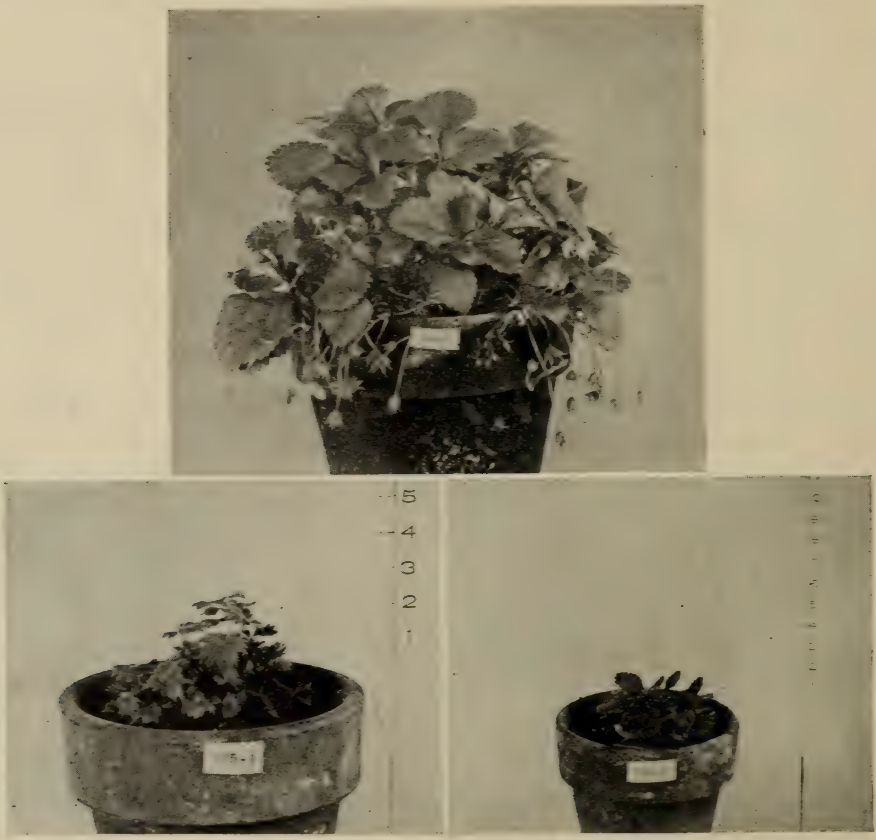

FIGURE 4.—*F. vesca*×*F. virginiana*, F_1. Above, vigorous but sterile hybrid; below, two dwarf hybrids.

F. vesca×*F. glauca*. Flowers of *F. vesca* pollinated with pollen from *F. glauca* set fruit readily, but as in the previous cross many of the achenes were poorly developed, and even the plump achenes germinated poorly. A sowing of 250 apparently normal achenes gave only 5 plants. One of the plants proved to be exactly like the female parent. It must have resulted either from pseudogamy or from accidental selfing. The other 4 are obviously hybrids. All are lacking in vigor. As in the *vesca-chiloensis* cross there is a conspicuous lack of uniformity in the population. Three of the plants are too small and weak to show any definite resemblance to either parent, and have never bloomed. The fourth plant is somewhat more vigorous, but still is considerably less vigorous than either parent. In color and texture the leaves resemble the *glauca* parent. It has bloomed rather freely but the flowers are small and abnormal in appearance. The petals are greenish; the stamens are rudimentary and never produce pollen. Numerous pollinations with pollen from the two parents have been without effect. Apparently the pistils are completely sterile.

F. vesca rosea×*F. glauca*. This cross may be made without difficulty but as in the previous case many of the resulting seeds were poorly developed. Germination of the plump seeds was only fair; a sowing of 76 seeds gave only 7 plants. Of these 5 are weak dwarfs with miniature leaves, which differ considerably among themselves. One produces a very large number of extremely minute leaves, giving the plant a fernlike appearance. None of these dwarfs has produced runners or flowers. The sixth plant is somewhat more vigorous; it produces many runners but it has never bloomed. The remaining plant, though undersized, produces flowers freely. They are very small, the anthers are rudimentary and produce no pollen, and the pistils have proved to be sterile with pollen from each parent. The color and texture of the leaves of this plant suggest the *glauca* parent.

F. vesca×*F. virginiana*. This cross regularly results in fruit setting, but many of the achenes remain small. Even those which appear plump and well developed, germinate poorly. From 500 seeds only 22 plants were obtained. Here again the F_1 population was extremely variable. Of the 22 plants 1 proved to be a pure *vesca*, like the female parent; whether it resulted from pseudogamy or accidental selfing is unknown. Eleven were miniature dwarfs. Several have died, the remainder are kept alive only with difficulty. They show considerable variability in color and leaf shape. None of these dwarfs has bloomed or produced runners. The remaining 10 plants are quite vigorous. They bloom freely but the flowers are small with rudimentary anthers which are quite sterile.

Pistil sterility also appears to be complete. A number of pollinations have been made with pollen from each parent without success. These 10 plants all resemble the *F. virginiana* parent quite closely, the dominance of the dark green color and leathery texture of the leaves as found in *F. virginiana* being particularly noticeable.

F. bracteata×*F. virginiana.* Fruit setting results quite regularly from this cross, but the number of seeds per fruit is small. A sowing of 24 seeds yielded 12 plants. Ten are true hybrids which resemble the *virginiana* parent much more closely than the *bracteata* parent, although there is a clear resemblance in leaf shape to the latter. The flowers are well developed and the anthers are almost normal in appearance. Occasionally a small amount of pollen is produced, which is mostly shrunken and abortive, though some grains appear to be normal. Selfing and backcrossing have thus far resulted in failure.

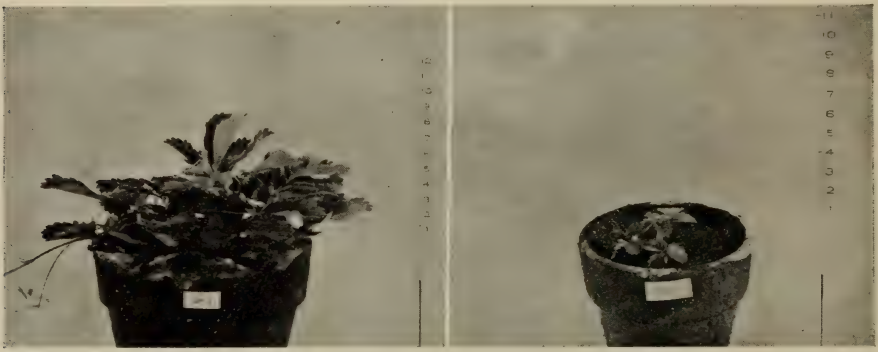

FIGURE 5.—*F. vesca*×*F. chiloensis*, F_1. At left, vigorous hybrid; at right, dwarf hybrid.

The other 2 plants present a most peculiar case. They are identical with the *bracteata* parent in appearance except that the flowers have no anthers; they are pistillate, whereas *F. bracteata* is hermaphroditic. True, isolated pistillate flowers have been observed on *F. bracteata* which may be the result either of fluctuation or of mutation, but they are rare. If these two plants are not true hybrids, they are the only purely pistillate individuals thus far encountered in the 7-chromosome group of species. Except for the anomalous pistillate flowers sometimes found on *F. bracteata* the group is hermaphroditic.

These two pistillates were crossed with *F. vesca rosea.* The progeny all proved to be hermaphroditic. It follows that the failure to develop stamens, if genetic, is due to the action of a recessive factor. The F_1 plants have been selfed but F_2 plants have not yet been grown.

Flowers of the 7-chromosome forms set fruit quite readily when pollinated with pollen from the 28-chromosome species, but many of the resulting achenes are poorly developed and even those which appear normal germinate poorly. Reciprocal crosses all resulted in failures.

The resulting F_1 populations are extremely variable. Three fairly distinct types may be recognized:

1. Vigorous hybrids, usually showing more or less complete dominance of the 28-chromosome type, with completely sterile flowers.
2. Weak miniatures and dwarfs, quite variable in appearance among themselves, which never flower.
3. Individuals of the maternal type. These appear only rarely. Whether they result from pseudogamy or from accidental selfing is not known.

Individuals intermediate between the first and second types are also occasionally found.

The 7-chromosome species crossed *inter se* give F_1 plants which show minor variations; the same is true of the 28-chromosome species. A possible explanation for the extreme variability of the hybrids between these two groups is that the variation in the gametes of the parental species resulting from their slight heterozygosity is enormously magnified in the F_1 zygote. Differences which are relatively insignificant in the economy of the pure species assume great importance in the hybrids. UPHOF (1922) reports similar F_1 variability in crosses between *Phaseolus vulgaris* and *Phaseolus multiflorus*. Since the variability is here even more difficult to explain because the two parental species are naturally self-fertilized and should therefore be quite homozygous, it is possible that in both cases chromosomal differences resulting from an elimination of one or more chromosomes in early zygotic divisions may be responsible for the differences in appearance.

Of these two explanations for the phenomenon as found in Fragaria, the first seems more plausible. A bit of evidence which appears to favor it is the following: *F. virginiana* and *F. glauca* are scarcely distinguishable from each other, and when crossed with other 28-chromosome species they usually give similar progenies; but there is a great difference between the F_1 populations which they produce when crossed with *F. vesca*. The former gives a certain percentage of vigorous, normal-appearing hybrids, while the latter has never given hybrids approaching either parent in robustness. Possibly differences exist between the two which, though of minor importance in crosses with other 28-chromosome species, are of great significance in hybrids between the two chromosome groups.

The possibility also remains that some of the dwarfs produced are haploids.

28-chromosome group × 21-chromosome group

F. virginiana × *F. elatior.* A pistillate form of *F. virginiana* was used as the female parent. It set fruit readily when pollinated with pollen from *F. elatior.* Most of the achenes were well developed and showed a high germination percentage, 100 seeds giving 90 plants. Here again the F_1 population was not uniform, although the variability was not nearly so great as in crosses between the 7-chromosome and 28-chromosome species. Some plants were very vigorous, others were relatively weak, with many gradations between the two extremes. The differences, however, instead of becoming more pronounced as the plants grew older, gradually diminished, and at the present time the population appears to be fairly uniform.

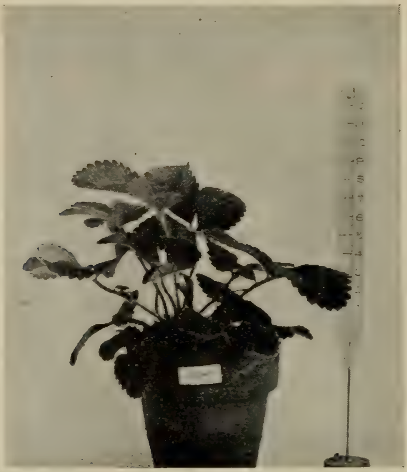

FIGURE 6.—*F. virginiana* × *F. elatior*, F_1. Practically indistinguishable from *F. elatior*, but sterile.

The dominance of the *elatior* type is striking. Many of the F_1 plants can scarcely be distinguished from the pure species. Its dense, shaggy pubescence, the rather pronounced venation of its leaves and the conformation of its flowers with their short styles and soft petals have been reproduced almost intact in the hybrids. Yet the hybrids appear to be completely sterile.

The dominant stamen-inhibiting factor, for which the female parent is heterozygous, apparently shows the same type of segregation in this cross as in intra-specific crosses, for though only a few plants have bloomed, both the staminate and the pistillate forms are represented. In addition to the true hybrids, some 15 plants of the maternal type appeared. Since the female parent was a pistillate, selfing cannot have occurred. However, at the time the cross was made other plants in the same isolation house were being pollinated with *virginiana* pollen; it is barely possible that contamination may have resulted from this source. The cross has been repeated under conditions which preclude the possibility of contamination. If plants of the maternal type appear in the resulting progeny, the occurrence of pseudogamy will be definitely established.

The "maternals" include both staminates and pistillates. It follows that if they have indeed resulted from pseudogamy, parthenogenesis rather than apogamy is responsible for their appearance, because the latter would produce plants identical with the mother—, that is, pistillates. The pistillate individuals which appeared if produced parthenogenetically should be homozygous for the dominant stamen-inhibiting factor. Several of them have been crossed with hermaphrodites to determine their constitution with respect to this factor. The outcome will answer the question as to their origin; if they prove to be heterozygous for the factor they must have resulted from contamination, if they are homozygous for the factor they must have been produced parthenogenetically.

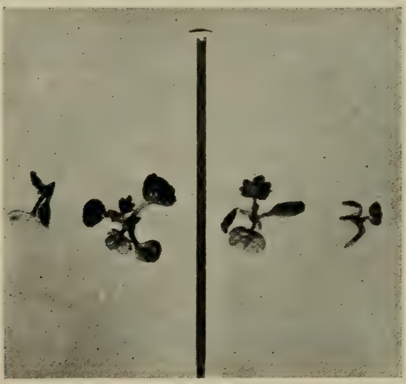

FIGURE 7.—*F. vesca*×*D. indica*, F_1. A common pin shows the size of these plants.

Generic crosses

F. vesca (7 chromosomes)×*Duchesnea indica* (42 chromosomes). Flowers of *F. vesca* pollinated with pollen from *D. indica* almost invariably set fruit. Many of the achenes, however, fail to develop normally. Germination is fairly high, 500 seeds producing about 30 plants. Four of these plants were apparently pure *F. vesca* and must have resulted either from accidental selfing or from pseudogamy. The remainder are evidently true hybrids. They are extremely small and weak. The leaves present a characteristic mottled appearance, due apparently to some abnormality in chlorophyll distribution. Many died before the end of the first month. In the course of that time they had developed as many as 15–20 miniature leaves. Four plants photographed at the age of five weeks are shown in figure 7.

F. vesca (7 chromosomes) × *Potentilla nepalensis* hybrid var. Miss Willmott (chromosome number unknown). This cross is difficult to make. Only one-third to one-half of the flowers pollinated set fruit, and each fruit bore only a few well developed achenes. A total of 141 achenes was obtained, which yielded two seedlings. Their color was a pale yellowish-green. In neither was there any indication of plumule development; both died within two weeks after germinating.

28-chromosome species crossed inter se.

The following crosses were made between species belonging to the 28-chromosome group:

F. chiloensis (Oregon No. 2) × *F. virginiana*
F. virginiana (pistillate) × *F. chiloensis* (Oregon No. 1)
F. virginiana (pistillate) × *F. glauca*

All of the above crosses are easily made. They gave in every case uniformly well developed achenes which showed a high germination percentage. The F_1 plants from all three crosses are vigorous, fairly uniform, fully fertile and intermediate between the two parents. The sex differences show normal segregation. They will be treated in another paper.

PARTHENOCARPY, PARTHENOGENESIS AND APOGAMY

During the summer of 1925 a group of pistillate plants of *F. virginiana* and *F. chiloensis* were isolated by placing them at the extreme end of one of the greenhouse benches. An assortment of plants of commercial varieties and of pure species were situated at the other end of the same bench some fifty feet away. The intervening space was filled with tall Nicotiana plants. In view of the absence of flower-visiting insects, the ventilators having been covered with mosquito netting, it was believed that the isolation was complete. During the months of March, April and May, these plants—all pistillates—developed no fruit except when artificially pollinated. In June, however, after a period of extremely hot weather, occasional seeds developed on flowers which, so far as was known, were unpollinated. It was believed that the high temperature, which appeared to be the only variable, might have been instrumental in stimulating apogamic development.

To obtain further evidence on the question fifteen individuals, including the commercial varieties Haverland, Warfield, Sample and Kellogg's Prize and the species *F. chiloensis* and *F. virginiana*, were placed in a glass chamber, the temperature of which was maintained at 40°–45°C. Each plant bore from 20 to 50 flowers, but not one showed the slightest trace of achene or receptacle development.

HABERLANDT (1921) was able in *Oenothera Lamarckiana*, by wounding the ovules in various ways, to induce the development of what he considers to be adventitious embryos. He found both bruising the ovules and piercing them with a needle to be effective in inducing the formation of these embryo-like structures. A number of receptacles of the isolated pistillate Fragaria plants were treated in a similar fashion, but in no case did the treatment result in an increased development,—indeed the receptacles usually degenerated the more rapidly as a result of the treatment.

In addition to the plants kept at a high temperature in the glass chamber, 125 pistillate plants—including *F. chiloensis*, *F. virginiana* and the commercial varieties Haverland, Hunterdown, Paul Jones, Kellogg's Prize, Big Late, Warfield and Portia—were isolated in a separate house and kept at ordinary greenhouse temperatures. The plants produced an average of not less than 25 flowers each, making a total of about 3000 flowers under observation. The receptacles of five flowers of Haverland, two of Warfield and two of Kellogg's Prize developed into berries, which were entirely normal in shape, color and texture, though only about one-fourth the usual size. The development, however, was confined to the receptacle—none of the achenes showed any increase in size. What the variables may have been which induced these flowers to further development while contemporaneous flowers on the same plant remained undeveloped, is not known. The position of the flowers on the cyme appears to be immaterial, since primary, secondary and tertiary flowers were represented. Two other flowers, these on a *F. chiloensis* plant, showed an aberrant behavior. When the flowers opened small terminal buds could be seen at the tips of the receptacles. These buds eventually produced several leaves which, though small, were entirely normal in other respects. The receptacles also underwent a considerable amount of development; they became fleshy and, in due time, passed through the normal ripening process, developing the color and aroma characteristic of the species.

One of these fruits was allowed to remain on the plant; the receptacle became fully ripe and finally decayed, resulting in the death of the little plant at its tip which had by this time produced three good leaves and had attained a height of about 2 centimeters. On neither fruit had the achenes undergone development.

These parthenocarpic fruits are of no significance from the genetic standpoint, except that they indicate that certain individuals show a greater tendency in this direction than others. As stated, in none of the eleven parthenocarpic fruits did the achenes undergo development, nor was any significant increase in size of achenes found in any of the 3000 flowers examined. It appears that spontaneous development of achenes in the 28-chromosome group, if it occurs at all under greenhouse conditions, is rare. It cannot be said, however, that it may not be encountered under other conditions or in other species of Fragaria. It is quite possible, too, that parthenogenesis and apogamy, if they do occur, as the rather frequent appearance of maternal plants in progenies resulting from species crosses might seem to indicate, are brought about only through the stimulus of penetration by the pollen tubes of foreign species. On this point these observations throw no light.

The fact that the "spontaneous" setting of seed observed on similar plants during the summer of 1925 failed to occur under more carefully guarded conditions, leads to the conclusion that the setting then obtained must have resulted from pollen carried by ants or by air currents. The extent of the danger of contamination from these two sources was not fully recognized at the time. It becomes more and more evident as the work progresses that the ordinary precautions to prevent contamination which have been found to be effective in other plants are not to be relied upon in Fragaria.

To determine whether pollination by other Rosaceæ is effective in bringing about development of the receptacles or achenes, nine plants of *F. vesca* were isolated, all the available flowers (from 10 to 15 on each) were emasculated, and each plant was pollinated with one of the following kinds of pollen: *Exachorda*, *Prunus*, *Chaenomoles*, *Rubus*, *Malus*, *Pyrus*, *Potentilla sterilis*, *Potentilla nepalensis* hybrid (var. Miss Willmott) and *Duchesnea indica*. Only the last two produced any visible development of the receptacle; flowers pollinated by the remainder withered at about the same time as did the unpollinated checks.

All of the flowers pollinated by *Duchesnea* produced good berries each with ten to twenty well developed achenes. Pollinations by *Potentilla nepalensis* resulted in three berries, each with two to five good achenes.

In the latter case the development of the receptacles was confined to the regions occupied by the achenes, as a result the berries were quite irregular in shape.

A large number of pistillate flowers of *F. virginiana* and *F. chiloensis* have also been pollinated with pollen from *Potentilla sterilis*, *P. nepalensis* and *Duchesnea indica.* Only the latter has proved effective in bringing about the development of fruit. The achenes are comparable in size to those resulting from pollination by the same species. The seeds are not yet ripe enough to test for germination, but it is to be supposed that here true fertilization has taken place.

The occasional spontaneous occurrence of parthenocarpy independent of achene development suggested the possibility that pollination with foreign pollen might produce a similar result. In every case, however, receptacle development was restricted to the regions immediately surrounding fertilized achenes.

INHERITANCE OF FLOWER COLOR AND FRUIT COLOR IN *FRAGARIA VESCA*

There is a fairly wide range of variability in leaf shape, leaf texture, pubescence, flower type, fruit shape and growth habit in Fragaria; but all of these characters appear to depend upon the interaction of a large number of factors, giving in F_1 an intermediate type, and in F_2 a series of gradations to which only an arbitrary classification can be applied. Only two characters, flower color and fruit color, and these only in the 7-chromosome group of species, were found to be simple and definite enough in their expression that their inheritance could be followed accurately.

The results of MILLARDET (1894) had suggested that Fragaria might present an exception to the usual rules of inheritance, or that the possible presence of parthenogenesis or apogamy might be expected to result in disturbed ratios. A study of the inheritance of flower color and fruit color was, therefore, undertaken to determine whether these two characters are inherited in a regular fashion, and whether they are independent or linked.

Flower color. The pink-flowered *F. vesca* used in the crosses is presumably identical with the type named by ROSTRUP (1888) *F. vesca rosea.* The pigment, a bright pink, follows the venation of the petals,—it is very pale at the edges and deepens toward the base, due to the convergence of the veins.

A plant homozygous for pink flowers and red fruit was crossed with one homozygous for white flowers and white fruit. Selfings and backcrosses of the F_1 plants thus obtained yielded data both on flower color and fruit color. Since the record for both characters is not complete for every plant, the data for the two characters are recorded separately.

The F_1 plants were all pink-flowered, the shade being slightly paler than that of the pink-flowered parent. Four of the F_1 plants were selected for further study, each being selfed and backcrossed reciprocally to the recessive.

The totals obtained from selfing, 128 pink and 46 white, deviate only 2.5 from the numbers expected on a 3:1 basis, indicating the action of a single factor pair, which may be designated *Pp*.

When the heterozygotes *Pp* were used as females and backcrossed to the recessive male *pp*, 89 pink and 116 white were obtained, showing a deviation from expectation on a 1:1 basis of 13.5—almost three times the probable error, 4.8.

When the heterozygotes *Pp* were used as males and backcrossed to the recessive female *pp* a slight excess of white-flowered individuals was also obtained, 111 white to 93 pink. The deviation from expectation, however, is only 9, less than twice the probable error, 4.8.

RICHARDSON (1918) made a preliminary study of the inheritance of pink flower color without reaching a definite conclusion as to its inheritance, apparently because of difficulty in classifying the progeny. By selfing a plant heterozygous for pink flower color he obtained 20 pink, 57 pale pink and 10 white, or nearly white. It appears that he failed, in classifying his plants, to distinguish between a pinkish blush suffused throughout the entire petals—a condition very frequently found, especially in the newly opened flowers—and the definite pink venation pattern resulting from the action of the factor under observation.

Fruit color. The white fruited form used in these studies was obtained from the UNITED STATES DEPARTMENT of AGRICULTURE under the name *F. americana alba.* It is a typical *vesca* except for the color of the fruit, which is creamy white. The seeds of the red-fruited form are of a dark mahogany color, those of the white fruited form are straw colored.

Red fruit color is almost completely dominant over white. The fruits of the F_1 plants were only a trifle lighter in color than those of the red-fruited parent. As in the flower color crosses, the four plants studied include selfings and reciprocal backcrosses to the recessive.

When the four heterozygotes were selfed 102 red-fruited and 56 white-fruited plants were obtained. On the basis of a 3:1 segregation the expected numbers are 118.5 and 39.5, the whites showing a considerable excess (16.5, or 4.4 times the probable error) over expectation. The reason for this excess is unknown, since the fact that the inheritance of the character is conditioned by the action of a single factor pair *Rr* is clearly shown by the backcross data.

When the heterozygotes *Rr* were used as females in backcrosses to the recessive male *rr*, 88 red-fruited and 91 white-fruited plants were obtained,—a very close approximation to the expected 1:1 ratio.

In the reciprocal of this cross, 84 red-fruited and 97 white-fruited plants were obtained. The expectation on a 1:1 basis is 90.5, the deviation 6.5 and the probable error 3.8.

Richardson (1914) obtained in the selfed progeny of a plant heterozygous for white fruit 70 red-fruited and 20 white-fruited plants—a very close approximation to a 3:1 ratio.

In Table 1 the relation of flower color and fruit color to each other is considered. The heterozygotes *Pp Rr* were here used as females with the double recessive *pp rr*. The parental classes, that is those with pink flowers and red fruit, show a total of 89, as against 90 in the recombination classes, a clear case of free recombination.

Table 1

Test for linkage between red fruit color and white flower color, heterozygote PpRr×double recessive.

FAMILY	PARENTAGE	*PR*	*Pr*	*pR*	*pr*
202	107-2×Double recessive	4	7	11	13
205	107-4× " "	15	21	21	23
208	107-6× " "	5	1	6	4
211	108-1× " "	14	11	12	11
Total		38	40	50	51

Total for parental classes	89
Total for recombination classes	90
Expected on basis of free recombination	89.5
Deviation	.5
Probable Error	4.5
Dev.÷P. E.	.1

Finally in table 2 are listed the results obtained when the heterozygotes *Pp Rr* are used as males in backcrosses to the double recessive *pp rr*.

The development of seeds without true fertilization, by means of apogamy or parthenogenesis, might be expected to result, in this cross, in an excess of individuals like the female parent, that is with white flowers and fruits. A slight excess was, in fact, obtained, there being 57 of this class as against 45 expected. The deviation from expectation is 12, the probable error 4. The figures suggest that parthenogenesis or apogamy may have been present, but the excess is hardly great enough to be significant. It must be remembered, also, that a similar excess could result from accidental selfing.

TABLE 2

Test for linkage between red fruit color and white flower color, double recessive × heterozygote PpRr.

FAMILY	PARENTAGE	*PR*	*Pr*	*pR*	*pr*
201	Double recessive × 107-2	5	8	10	7
204	" " × 107-4	8	6	4	6
207	" " × 107-6	11	11	16	24
210	" " × 108-1	13	16	17	19
Total		37	41	47	56

Total for parental classes	93
Total for recombination classes	88
Expected on basis of free recombination	90.5
Deviation	2.5
Probable Error	4.5
Dev. ÷ P. E.	.6

A test for the presence of pseudogamy, that is, the parthenogenetic or apogamic development of seed brought about through the stimulus of pollen tube penetration, may be made by pollinating a recessive form under the most carefully guarded conditions with pollen from a homozygous dominant. Even under these circumstances the appearance of recessives in the progeny cannot be regarded as positive proof of pseudogamy, since mutation in the pollen to the recessive factor would produce a similar result. If, however, two factors instead of one are followed, this difficulty is eliminated, the likelihood of a simultaneous mutation in both factors being too remote to be considered. The most serious danger of error in such a test is, of course, that of accidental selfing. The significance of the results obtained must depend largely upon how carefully this danger is guarded against.

A preliminary test carried out according to the plan just described has been made on a small scale. A homozygous recessive white-flowered, white-fruited individual was pollinated with pollen from a homozygous dominant pink-flowered, red-fruited individual. The resulting progeny (Family 215) contained 27 individuals all of which were pink-flowered and red-fruited. The fact that no recessives appeared, however, does not justify any positive statements as to the presence or absence of pseudogamy. Different results might be obtained under different environmental conditions. Furthermore, the number of plants under observation is too small to be very significant. It is also quite possible that parthenogenesis or apogamy, if they occur in Fragaria as indicated by our other crosses, result only when true fertilization is rendered difficult by the use of pollen from more distant species.

MILLARDET'S "FAUX HYBRIDES"

Few cases of anomalous inheritance are cited more frequently than the Fragaria hybrids of MILLARDET. In fact, it was the exceptional nature of his results that led to our re-examination of the behavior of species hybrids in this genus.

MILLARDET'S investigations, carried on at Bordeaux, where he occupied the botanical chair on the Faculté des Sciences, were begun in 1883 and extended over a period of eleven years. The thesis to which his observations led him is concisely stated in the introduction to his paper in the following words:

> "Dans son important ouvrage sur l'hybridation des végétaux, Gärtner formule cette loi que "nous ne connaissons aucun cas où le type d'une des espèces composantes d'un hybride ait passé intégralement dans ce dernier'. Tous les physiologistes qui se sont occupés d'hybridation, avant comme après lui, ont partagé cette même opinion. Mon intention, dans ce travail, est de démontrer que dans le genre Fraisier (*Fragaria*) les produits obtenus par l'hybridation de certaines espèces font exception à la règle générale qu'on vient de lire. Ils reproduisent intégralement le type spécifique du père ou celui de la mère, et ressemblent par conséquent exclusivement soit à l'un, soit à l'autre, sans réunir jamais à la fois aucun des caractères distinctifs des deux espéces composantes."

The results of the crosses upon which he bases his conclusions will be discussed in the order in which he presents them.

The first cross described is one in which "Fraisier des quatre saisons blanc" is used as the female parent and "Chili velu" is used as the male parent. The female parent, according to his description, is *F. vesca*, variety *alba;* the male parent is an hermaphroditic variety of *F. chiloensis* He obtained 4 hybrids, of which 1 resembled the female parent in every respect except that the fruit was *red;* the others resembled the male parent "from which it was almost impossible to distinguish them."

The *vesca* group, to which the female parent belongs, has 7 pairs of chromosomes; *F. chiloensis*, the male parent, has 28 pairs. In our own progenies from crosses between these two groups there have appeared individuals which displayed a marked dominance in F_1 of the 28-chromosome type over the 7-chromosome type. The dominance is not complete for every character, certain features such as flower form, for example, being intermediate between the two parents; but that MILLARDET should have seen in these three plants a "reproduction du type specifique paternel" is not surprising.

Even in the absence of other evidence, it is fairly safe to conclude from the partial sterility of these three plants that they are true hybrids.

> "Les fleurs en sont grandes, hermaphrodites, mais a étamines assez peu dévelopées. Les anthères sont à peu près normales ainsi que le pollen. . . . . Toutes trois sont fertiles, mais médiocrement."

Our own hybrids between these two groups have thus far proved to be completely sterile. RICHARDSON (1918), however, obtained a slight degree of fertility in hybrids between *F. vesca* and *F. virginiana*, a cross involving the same chromosome numbers as the one under discussion.

The progenies of these 3 plants gave further evidence of the hybrid sterility which is to be expected in crosses between the 7-chromosome and the 28-chromosome group. Thus sowing "*R*" produced 2 plants "du type *chiloensis* pur. Ces plantes ont toujours été infertiles. Étamines et anthères peu développées. Pollen imparfait ou nul." MILLARDET stresses the fact that the plants are "paternal" in type, their sterility he evidently considers incidental and of no particular significance.

Several other sowings were made from these 3 plants, mostly from open pollinated seed. MILLARDET emphasizes the fact that in none of these progenies was there to be found the "slightest trace" of *vesca* characters. No conclusions can be drawn from these progenies since the pollen parents are unknown. If the pollen were supplied by one or more species of the 28-chromosome group, the *vesca* characters would again be covered up.

Let us return to the first of the 4 hybrids which MILLARDET describes as being "exactement semblable a la mère, sauf les fruits, qui sont rouges." The fact that this plant was red-fruited, while its mother was white-fruited does not trouble MILLARDET. In speaking of this family in another place, he says

> "dans l'exemple 1, sur quatre hybrides, tandis que trois reproduisaient le type du père, un reproduisait celui de la mère, avec cette variante *sans importance* que les fruits au lieu d'être blancs étaient rouges." (The italics are our own).

It will be recalled that fruit color in *F. vesca* depends upon the interaction of a single pair of factors, white being recessive to red. Red-fruited individuals have the constitution *RR* or *Rr*, white-fruited, *rr*. The appearance of a white-fruited individual in the progeny of a red-fruited plant might be explained without much difficulty since the mother could have been heterozygous for the *R* factor; in such a case white-fruited offspring resulting from the parthenogenetic development of reduced eggs would have been at least a theoretical possibility. Even mutation as an explanation might have some plausibility if the change had been from red to white, for loss of color is rather common in plants; but there are few cases in the literature of white mutating to color. Apparently the reaction is not easily reversible.

Assuming that the plant is really of the parentage which MILLARDET assigns to it, only one explanation suggests itself for the red-fruit color (except the improbable one of mutation). Had it received the full complement of 28 chromosomes from its male parent, *F. chiloensis*, it would have shown the dominance of *chiloensis* characters, as did the other three members of the progeny. ICHIJIMA (1926) has found that at the reduction division of the hybrids between the 7-chromosome and 28-chromosome groups, 7 bivalents and 21 uni valents can be distinguished. It appears that the 7 *vesca* chromosomes pair with 7 of the 28 chromosomes from the other parent, leaving 21 unpaired. It is conceivable that at the first division following the fusion of the male and female nuclei the unpaired chromosomes might be left behind, resulting in an individual with 7 pairs of chromosomes, like the female parent in appearance but having red fruit. That a plant receiving such a complement of chromosomes would appear *vesca*-like is purely an assumption, nor is there any evidence on the question as to whether the unpaired chromosomes might be left behind at the first division of the newly formed zygote, as postulated.

There is, however, a bit of evidence which might lend credence to the possibility. The F_1 individuals resulting from species crosses of this type are extremely variable, ranging from vigorous plants entirely normal in appearance except for their sterility, to tiny dwarfs which can be kept alive only with difficulty. Varying degrees of chromosome elimination might conceivably bring about such results as those which MILLARDET obtained. But since no case has been found agreeing in every detail with that of the plant under discussion, the likelihood that the explanation suggested is the correct one appears to be very remote.

It seems much more probable that this red-fruited *vesca* plant is the result of one of several possible forms of contamination. The seed may have been present in the soil in which the sowing was made, *F. vesca* with the red fruits being the common wild strawberry of France. MILLARDET states that all sowings were made in "terre de bruyère" procured directly from the forest. This practice may have reduced the danger of contamination from foreign seed, but it certainly cannot have removed it entirely.

Another possible source of error comes from trespassing runners. Unless the plants are kept constantly under observation a runner from a neighboring plant may, in a very short time, become established on the spot left vacant by the death of the original plant, and once the connection of the intruder with its parent is broken, as usually happens when the bed is cultivated, the evidence as to its origin is destroyed. A third and most probable source of contamination is that of wind-borne pollen. MILLARDET recognized the possibility of contamination from this source, but apparently he did not regard it as serious. He describes the cages used to protect the plants as being

> "garnies de tulle, et quelquefois même de mousseline très fine, de manière à empêcher complètement non seulement l'accès des insectes, mais aussi, lorsqu'elles étaient couvertes de mousseline, la pénétration du pollen étranger qui aurait pu être apporté par le vent."

Certainly the cages which were covered with tulle offered practically no protection, and it is questionable whether those covered with muslin would exclude entirely pollen grains as small as those of Fragaria.

Most of the remaining crosses reported by MILLARDET present difficulties of interpretation similar to those just discussed. It is hardly desirable to consider each cross in detail. The results which he obtained will be briefly set down, however, as a matter of record.

"*Hybridation No. 10.*" Black Hautbois (*F. elatior*—21-chromosome group—the parenthesized explanatory notes being our own) × Globe (cultivated variety—28-chromosome group). Fifteen plants were grown from the cross; 14 were exactly like the female parent, 1 was exactly like the male parent.

"*Hybridation No. 8.*" Fraisier des quatre saison blanc (*F. vesca*—7-chromosome group) × Globe (cultivated variety—28-chromosome group). Only 1 plant resulted from this cross. MILLARDET describes it thus:

> "La plante est vigoureuse et fleurit abondamment. Les fleurs sont petites (de la grandeur des fleurs femelles de *Virginiana* et *Chiloensis*), à étamines atrophiées et à anthères de couleur sale, ne contenant pas de pollen. Comme la plante s'est toujours montrée stérile, on doit en inférer que ses ovules ne sont pas en meilleur état que les anthères. Dans cet exemple, l'hybridation a reproduit le type spécifique paternel sans aucun mélange des caractères du type maternel, mais il y a impuissance complète tant des organes femelles que des mâles."

The plant is obviously a true hybrid with the characters of the 28-chromosome species dominant, as is usually the case in crosses between these two groups.

"*Hybridation No. 16.*" Fraisier des Alpes (*F. vesca*—7 chromosome group) × Ananas (cultivated variety—28-chromosome group). This cross yielded 24 plants, of which all but 1 were exactly like the female parent. The exceptional plant

> "reproduit le type paternel sans mélange de caractères étrangers à ce type. Mais cette plante est un peu rabougrie; elle fleurit peu et n'a jamais porté de fruits. Les fleurs sont petites, à étamines et anthères atrophiées, tout à fait semblables aux fleurs de la plante dont il vient d'être question (hybride 8)."

This plant, like the one in the preceding cross, is undoubtedly a true hybrid with dominance of the paternal characters.

"*Hybridation No. 1.*" Belle Bordelaise (*F. elatior*—21-chromosome group) × Gaillon rouge (*F. vesca*—7-chromosome group). The family consisted of 3 plants, all like the female parent. They were fertile, and therefore must have resulted either from induced parthenogenesis or apogamy, or from contamination.

"*Hybridation No. 2.*" Black Hautbois (*F. elatior*—21-chromosome group) × Docteur Nicaise (cultivated variety—28-chromosome group). This family consisted of 8 plants, all like the female parent. Again there is pseudogamy or contamination.

"*Hybridation No. 3.*" Black Hautbois (*F. elatior*—21-chromosome group) × Chili velu (*F. chiloensis*—28-chromosome group). The family consisted of 8 plants, all like the female parent. Again there is pseudogamy or contamination.

"*Hybridation No. 4.*" Black Hautbois (*F. elatior*—21-chromosome group) × Gaillon Rouge (*F. vesca*—7-chromosome group). The family consisted of 10 plants, all like the female parent, and which, therefore, must have arisen through pseudogamy or through contamination. Our own crosses between the 7-chromosome group and the 21-chromosome group gave hybrids which never developed beyond the seedling stage.

Open pollinated seed from these hybrids gave, besides maternals, 2 plants of the 28-chromosome type, which presumably was due to the dominance of the characters transmitted through pollen from plants of that type. In addition 1 plant showing pure *vesca* characters appeared. Contamination in the seed pan or the trespassing of runners is the only explanation that can be offered for this plant. CORRENS (1901) comments on it thus:

"Ich weiss erst seit ein paar Wochen privatim, durch die Liebenswürdigkeit MILLARDET'S, dass sich hier ein Irrthum eingeschlichen hat. . . . . Es waren mit der zur Cultur verwandten Haideerde Samen der wilden *Fragaria vesca* eingeschleppt worden. Herr Professor MILLARDET hat mir gütigst erlaubt, dies zu veröffentlichen."

"Hybridation No. 13." Black Hautbois (*F. elatior*—21-chromosome group) × Docteur Nicaise (cultivated variety—28-chromosome group). 3 plants, all maternal.

"Hybridation No. 14." *F. elatior* (21-chromosome group) × Ananas (cultivated variety—28-chromosome group). 9 plants, all maternal.

"Hybridation No. 17." *F. elatior* (21-chromosome group) × *F. virginiana* (28-chromosome group). 9 plants, all maternal.

"Hybridation No. 20." *F. vesca* (7-chromosome group) × *F. elatior* (21-chromosome group). 5 plants, all maternal.

"Hybridation No. 23." Fraisiers des Alpes (*F. vesca*—7-chromosome group) × *F. elatior* (21-chromosome group). 3 plants, all maternal.

"Hybridation No. 25." Hybride 11–3 × *F. elatior* (21-chromosome group). 2 plants, all maternal.

"Hybridation No. 35." Docteur Nicaise (cultivated variety—28-chromosome group) × *F. vesca* (7-chromosome group). 4 plants, all maternal.

"Hybridation No. 39." *F. vesca* (7-chromosome group) × *F. virginiana* (28-chromosome group). 1 plant, maternal.

"Hybridation No. 45." *F. californica* (7-chromosome group) × Docteur Nicaise (cultivated variety—28-chromosome group). 9 plants, all maternal.

"Hybridation No. 46." *F. vesca* (7-chromosome group) × *F. californica* (7-chromosome group). 6 plants, all maternal.

One may sum up MILLARDET'S results as follows: He made many crosses between different species of Fragaria using methods of control which are satisfactory for most plants, but which we have found unsatisfactory for Fragaria where the pollen is extremely small. He dealt with general resemblances in species which differ from each other by rather vague quantitative characters, and was not impressed with minor, individual characters, with dwarfness, or with sterility, as genetic evidence. Many of his populations came from open-pollinated plants. Some of the aberrant plants which appeared, therefore, almost certainly resulted from pollen, seed, or runner contamination.

In addition, there are two phenomena to be explained. In the first place, many plants appeared which were of the type of the female parent.

These maternal plants may have resulted either from accidental pollination with pollen of the same general type, or from pseudogamy. We are not prepared at present to accept either alternative to the exclusion of the other, but it cannot be denied that on general grounds pseudogamy is a possibility. In addition, there are four cases where individuals were obtained which resembled the male parent, excluding two instances of open pollination where the male parent was unknown. In two of these cases, *F. vesca*×*Globe* and *F. vesca*×*Ananas* (7-chromosome type×28-chromosome type), the exceptional individuals, two in number, showed their hybrid nature by being completely sterile. The other two cases were *F. vesca*×*F. chiloensis* (7-chromosome type×28-chromosome type) and *F. elatior*×*Globe* (21-chromosome type×28-chromosome type). In each instance these plants showed their hybrid nature by greatly reduced fertility. They did indeed yield a few F_2 progeny from open-pollinated seed, which were somewhat variable, but which in general resembled the plants of the F_1 generation. These results, therefore, show only the dominance of the type with the higher chromosome number which is usually to be expected when plants are crossed which have markedly different chromosome numbers, and the unequal distribution of characters which naturally occurs under such circumstances. On the other hand, the case where the 28-chromosome type dominates the 21-chromosome type is in direct opposition to our own results. In our experience, the characters of the 21-chromosome type dominate the characters of the 28-chromosome type as well as the characters of the 7-chromosome type; but this is contrary to the experience of investigators on other genera with crosses showing analogous chromosome differences.

The results obtained by Solms-Laubach (1907) have been cited more than once as confirming the conclusions of Millardet. Indeed, it appears that Solms-Laubach himself believed that such was the case. He speaks of his findings as being "in schönster Uebereinstimmung mit den Ergebnissen jenes Autors."

Solms-Laubach made several crosses between a pistillate form of *F. virginiana* as the female parent and *F. elatior* as the male parent. The heavy pubescence, the shape of the leaves, their light, slightly greyish green color, their deeply impressed venation and the general growth habit of *F. elatior* have proved in our own crosses to be almost completely dominant over the corresponding characters in *F. virginiana*. Solms-Laubach obtained a similar result, his experience being therefore different from that of Millardet.

> "Aus den Samen der *F. virginiana* war also hier fast ganz reine *elatior* (moschata) auf gegangen, es war 'fécondation sans croisement' in schonster Form eingetreten."

The plants which result from crosses between these two species are completely or almost completely sterile; SOLMS-LAUBACH observed the fact but appears to have attached no significance to it.

It is obvious that here again we are dealing with true hybrids showing a dominance of the paternal type and displaying the sterility characteristic of hybrids between species differing in their chromosome numbers.

GIARD (1903) interprets the patroclinous hybrids of MILLARDET as having a basis similar to that of the development of spermatozoa in enucleated sea urchin eggs as observed by BOVERI (1895) and DELAGE (1899). This hypothesis is almost certainly incorrect. ICHIJIMA (loc.cit.) has made chromosome counts on several of such patroclinous hybrids and finds that they contain the sum of the haploid numbers of the parental species, indicating that the nuclei of both hybrids participated in the formation of the zygotic numbers.

The matroclinous plants which constituted a large part of most of MILLARDET'S progenies GIARD believes have resulted from a parthenogenetic development of the egg, induced by stimulus from the foreign pollen tube. They may have originated in this way, but it must be kept in mind that they may also have resulted from accidental selfing. In our own crosses between different species, diploid individuals of the maternal type have appeared from time to time. Furthermore, apogamy is a regular occurrence in other Rosaceæ, notably in *Rosa* (TACKHOLM, 1922 and LIDFORRS, 1914). It seems significant, however, that with the adoption of more rigorous measures to prevent contamination, such individuals have appeared less and less frequently in our cultures.

The most recent reference to the work of MILLARDET is that of LONGLEY (1926). He describes the results of a cytological study of various Fragaria forms, among them one matroclinous and one patroclinous hybrid, which he apparently believes afford further evidence in favor of MILLARDET'S assumption that a unique form of inheritance obtains in Fragaria.

The matroclinous plant appeared in the progeny of a cross between *F. vesca* (7-chromosome group) and *F. chiloensis* (28-chromosome group). This plant was found by LONGLEY to contain 7 bivalent chromosomes—the same number as in the female parent. This observation, however, throws no light on the question as to whether such plants have resulted from parthenogenesis or from accidental selfing.

The second plant appeared in the progeny of a cross between *F. vesca* var. *alba* (7-chromosome group) and the cultivated variety Aroma (28-chromosome group). It resembles the male parent morphologically, and was found by LONGLEY to have the same chromosome number. Neither of these facts is particularly significant in establishing whether the plant is actually the product of the cross from which it is supposed to have originated. Here again the question arises as to whether the sowing was made in sterile soil, and whether the plant was kept constantly under observation from the seedling stage until maturity. Nevertheless, the possibility that the chromosomes of the female may somehow be lost at the fusion of the two gametes, resulting in an individual which duplicates the male parent, is certainly not to be excluded. The Tripsacum-Euchlaena hybrid of COLLINS and KEMPTON (1916) which, both in appearance and in three generations of breeding, showed no trace of any of the characters of the female, appears to furnish an example of some such phenomenon.

CONCLUSIONS

Fragaria species having the same number of chromosomes cross freely, yielding perfectly fertile hybrids in which segregation takes place in the normal manner. Typical segregation in sex manifestations was observed in crosses between 28-chromosome species, while normal segregation of two independent pairs of allelomorphs, pink flowers and white flowers and red fruit and white fruit, was observed on fairly large populations in crosses between 7-chromosome species.

Crosses in which *F. elatior*, the 21-chromosome species, was used as the female and 7-chromosome species as the male, resulted in failure. In the reciprocal crosses, fruit set readily; but the seeds showed only about 0.7 percent germination, and the few seedlings obtained died within two weeks.

Crosses in which the various 28-chromosome species were used as the female and the various 7-chromosome species as the male also failed; but reciprocal crosses were made without difficulty. Many of the achenes were poorly developed and germination was low. Three types of plants appeared in the F_1 generation; vigorous hybrids showing a more or less complete dominance of the 28-chromosome type, dwarfs which did not flower, and maternals. The hybrids were completely sterile.

A pistillate individual of the 28-chromosome group set fruit readily with pollen from *F. elatior* (21-chromosomes). The seeds germinated well (90 percent). The plants were variable in vigor, but showed a striking dominance of the *F. elatior* characters. They were completely sterile. Maternals were also obtained.

The flowers of 7-chromosome species set fruit readily with pollen from *Duchesnea indica* (42 chromosomes). From 500 seeds, 30 plants were obtained. Of these 24 were small, weak hybrids; the remainder were maternals.

Two seedlings were also obtained from *F. vesca* (7 chromosomes) flowers fertilized with the pollen of *Potentilla nepalensis*.

Though rare cases of parthenocarpy were observed, pseudogamy was not induced by treatments given, unless the maternal individuals appearing in the crosses between species differing in chromosome number are such. These maternals have the diploid number of chromosomes characteristic of the species involved, and may result either from induced parthenogenesis (haploid development) with a doubling of the chromosomes during early development, or from induced apogamy (diploid development). But since the pollen in all the Fragaria species is extremely small and easily carried by wind currents, we are not prepared to maintain that these plants did not result from accidental selfing.

MILLARDET'S plants of maternal type must have been, like our own, the result either of pseudogamy or of selfing. His plants of paternal type were all obtained by crossing species with a lower chromosome number with species with a higher chromosome number, and were, we believe, merely hybrids showing general dominance of the type with the higher chromosome number. There is no evidence, therefore, that inheritance in Fragaria is of a new or unusual kind as has been so often maintained.

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GENETICS, JULY, 1927

TABLE OF CONTENTS

PAGE

MANGELSDORF, A. J., AND EAST, E. M. Studies on the genetics of Fragaria....307

DANFORTH, C. H., AND DE ABERLE, S. B. The functional inter-relation of certain genes in the development of the mouse....340

BRINK, R. A., AND BURNHAM, C. R., Differential action of the sugary gene in maize on two alternative classes of male gametophytes....348

PAINTER, THEOPHILUS S., The chromosome constitution of Gates' 'non-disjunction' ($v=o$) mice....379

[This number of GENETICS (12: 307-392) was issued August 9]

THE GENETICS AND PHYSIOLOGY OF SELF-STERILITY IN NICOTIANA

By

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and

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NEW YORK

1927

Reprinted, without change of paging, from MEMOIRS OF THE HORTICULTURAL SOCIETY OF NEW YORK **3**: 321-323. July 1927.

THE GENETICS AND PHYSIOLOGY OF SELF-STERILITY IN NICOTIANA

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Although investigations have been under way on the problem of self-sterility at the genetics laboratory of the Bussey Institution of Harvard University for the past fifteen years, using *Nicotiana alata* and *N. Sandaræ* as material, it was only recently that sufficient genetic tests had been made to permit the formulation of a theory that interpreted satisfactorily the behavior of self-sterile plants in crosses where only self-sterile individuals were involved. This theory was published in the Proceedings of the National Academy of Sciences* in February, 1925, but since it is not generally known, it may not be out of place to outline it here.

In endeavoring to analyze the mechanism which controls the behavior of these self-sterile plants, the chief obstacle was the difficulty of obtaining sufficient quantities of selfed seed, because only by the use of seedlings obtained by selfing can certain essential tests be made. When, a few years ago, it was found that nearly all of these particular self-sterile plants could be selfed by pollinating very young buds, therefore, it was felt that the strictly genetic problem of self-sterility stood some chance of being solved. It had previously been found that self-incompatibility and cross-incompatibility were due simply to a slow rate of pollen-tube growth and not to a true incompatibility between the gametes which would prevent zygote formation after the male nucleus had entered the micropyle of the embryo sac.

By taking advantage of this procedure, therefore, numerous families were produced among the so-called *Sanderæ* hybrids which consisted of two groups of progeny, all members of each group being sterile with each other but fertile with all members of the other group. A large series of crosses was made between the members of these various classes. The results obtained were similar. Let us take, for example, three classes which we may call A, B and C. Class A ♀ × Class B ♂ produced equal numbers of plants of Class B and Class C. The reciprocal cross, Class B ♀ × Class A ♂, yielded an equal number of plants of the two classes, A and C. Similarly, Class A ♀ × Class C ♂ gave an equal number of plants of Classes B and C. The reciprocal cross gave an equal number of plants of Classes A and B. A large number of such crosses, yielding without exception the same type of result, led us to the conclusion that the behavior of these three classes could be accounted for under the assumption that three members of one allelomorphic system, S_1, S_2 and S_3, here control the phenomenon of self-sterility. Class A is S_1S_3,

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*East, E. M., and Mangelsdorf, A. J. A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. 11: 166-171. 1925.

Class B is S_1S_2 and Class C is S_2S_3. If, then, it is assumed that a plant affords stimulus only to pollen which bears sterility factors other than those found in its own tissues, all the observed facts are satisfied.

The ordinary type of plant found in self-sterile cultures is naturally heterozygous for the self-sterility factors carried; and very often when dealing with related plants, one sterility factor is common to both plants. A plant S_1S_2 may be crossed with a plant S_1S_3. If, then, in the competition which ensues among the pollen grains of plant S_1S_3 those bearing the factor S_3 grow much faster than those bearing the factor S_1, equal classes of zygotes will be produced having the constitution S_1S_3 and S_2S_3. This is, indeed, what is found. The reciprocal cross, however, $S_1S_3 \times S_1S_2$, results in equal-sized classes which prove to be S_1S_2 and S_2S_3. In other words, when both plants are heterozygous and one sterility factor is common to both, the results of reciprocal crosses are unlike and the class of the mother is never obtained.

It may happen, however, that the self-sterility plants used in matings have no sterility factor in common; in such a case, the results of a reciprocal cross are identical. For example, when a plant S_1S_2 is crossed with a plant S_3S_4, four equal-sized classes are produced which can be proved by proper testing to have the formulæ S_1S_3, S_1S_4, S_2S_3 and S_2S_4. Each of these four classes is different from the class of either parent and is, therefore, fertile reciprocally with them.

A very interesting and critical test can be devised to prove or disprove this hypothesis. If a heterozygous plant—for example, a plant having the formula S_1S_2—is fertilized with its own pollen in the very young bud, there will be no competition between the pollen-tubes because the two types grow equally slowly; yet seed will be obtained, owing to the extraordinary length of time for pollen tube growth, and owing possibly also to the fact that pollen tubes grow more rapidly in young bud pollinations of this species. Ordinary Mendelian recombination should occur, therefore, yielding homozygous S_1S_1 and S_2S_2 plants, as well as the parental class S_1S_2. Plants of the two homozygous types should be fertile together, and should give the same type as that of the mother when crossed together. Each of these homozygous classes should be fertile as females with the heterozygous class S_1S_2, yielding a single class S_1S_2, but both homozygous classes should be sterile with the heterozygous class when used as males. The reason for this odd result is perfectly simple, according to the hypothesis. The homozygous S_1S_1 plants will cross reciprocally with S_2S_2 plants because the factors are unlike and every individual produced will have the formula S_1S_2. Naturally, however, neither the S_1S_1 pollen nor the S_2S_2 pollen will function on the heterozygous plants S_1S_2 because in each case like factors are involved. On the other hand, in the reciprocal crosses $S_1S_1 \times S_1S_2$, or $S_2S_2 \times S_1S_2$, the entire resulting progeny will belong to the Class S_1S_2 because in the first case only S_2 pollen functions and in the second case only S_1 pollen functions.

All of these tests have been made and have fulfilled expectation in a really remarkable way. Seven allelomorphs have been isolated and homozygous "testers" produced which can be, and have been, used in further tests. That these factors are all members of one allelomorphic system—that is to say, that they are mutations at one locus—is shown first by the fact that when any two enter a given cross, only those two are recovered, and second by the fact that each is linked in inheritance with a certain flower-color factor and gives similar crossover values. Thus far no second locus, effective in self-sterility, has been found. We may, therefore, assume either that *like* factors produce substances that inhibit pollen-tube growth, or that *unlike* factors produce substances that accelerate pollen-tube growth. For various reasons the second assumption seems more probable; but speaking philosophically, if only one allelomorphic series of factors affecting pollen-tube growth is involved, it makes no difference which interpretation is adopted.

We have been greatly interested to find that the various data of Erwin Baur on self-sterile species of *Antirrhinum* yield to an analysis with the postulates given above. The recent work of Professor Ernst Lehmann and the work of Professor M. J. Sirks on *Veronica syriaca* and *Verbascum phoeniceum* respectively—reported in the proceedings of this conference—also fall into line. Doubtless cases of self-sterility will be found which do not yield so readily to analysis. Perhaps real differences of mechanism will be found; but since plants so different as *Nicotianas, Antirrhinums, Veronicas* and *Verbascums* show behavior which accords with the theory outlined above, we are inclined to feel that seemingly anomalous behavior in other genera is more likely to be due to additional variables being involved than to fundamental differences in the controlling mechanism.

THE INHERITANCE OF HETEROSTYLY IN *LYTHRUM SALICARIA*

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Harvard University, Bussey Institution, Forest Hills, Massachusetts

Reprinted from GENETICS **12**: 393-414, September, 1927

GENETICS

A Periodical Record of Investigations Bearing on
Heredity and Variation

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Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879.

THE INHERITANCE OF HETEROSTYLY IN *LYTHRUM SALICARIA*

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Received February 5, 1927

TABLE OF CONTENTS

PAGE
INTRODUCTION 393
Experimental work 396
Self-fertilizations 397
Crosses between Long styled and Mid styled plants 400
Crosses between Long styled and Short styled plants 401
Crosses between Mid styled and Short styled plants 405
DISCUSSION 407
SUMMARY 414
LITERATURE CITED 414

INTRODUCTION

The biology of those plants termed heterostyled by HILDEBRAND was brought into prominence by the publication of DARWIN'S work, "The different forms of flowers on plants of the same species," which appeared first in 1877, and was revised in 1880 and 1884. Darwin was interested in the phenomenon, which is rather widespread, from its bearing on certain problems of evolution; and he carried out a really extraordinary amount of experimental work on the subject, if one makes due allowance for the state of his health at the time.

With his habitual thoroughness, he made numerous observations on a great variety of plants, and established the fact that heterostyly is the extreme manifestation of a graded polymorphism found in various plants usually described as homostyled. These homostyled plants exhibit their weak dimorphism by bearing anthers which differ in color, or by producing pollen differing in color or size. The anthers may also be different in structure. His most extensive experiments, however, were concerned with various dimorphic species of Primula and with trimorphic species of Lythrum and Oxalis.

Though true heterostyly shows variability, like everything else in Nature, the type of polymorphism shown is usually similar in related species. Conditions in *Primula veris*, for example, can be taken as typical of the genus. Here the long styled plants have globular stigmas with long papillae; they produce oblong pollen measuring .0254 mm at their greatest diameter; they flower earlier than the short styled type, and produce less seed. The short styled flowers have stigmas depressed at the summit, with shorter papillae and bear pollen .038 mm in diameter.

Generally speaking—*Primula auricula* formed an exception—Darwin found that the mating Long×Short was the most fertile. Short×Long exhibited the next highest fertility. The illegitimate mating Long×Long showed a considerably diminished fertility, while the illegitimate mating Short×Short was least fertile of all. The offspring of Long×Long was practically always Long, though in experiments with *Primula veris* 6 short styled plants (probably due to experimental error) were obtained out of a population of 162. Short×Short yielded both Longs and Shorts; the number of plants obtained, however, was too small to give a dependable ratio.

In *Lythrum salicaria*, Darwin established a number of interesting facts. The long styled form has a globular stigma larger than those of the other two forms and with longer papillæ. The anthers of the mid-length set are larger than the others. The pollen is the same color in both sets, but that of the mids is larger than that of the shorts. The mid styled form has a long set of stamens with pink filaments bearing purple anthers and green pollen, and a short set with greenish filaments bearing greenish anthers and yellow pollen. The green pollen is larger than the yellow pollen. The short styled form has two sets of stamens much like those of the mid styled form, and bearing like it, green and yellow pollen respectively. The size of the seeds of the three forms is such that five seeds from long styled plants very nearly equal six seeds from mid styled plants or seven seeds from short styled plants.

The legitimate unions, which are cases in which the stigmas are pollinated with pollen from anthers of other flowers borne at the same height, were found to be more fertile than the other or illegitimate unions. Illegitimate unions where long styled or short styled plants were used as mothers were too unproductive to make averages worth while, but mid styled forms fertilized by pollen from the short stamens of the long styled form or by the pollen from the long stamens of the short styled form proved to be almost as productive as legitimate unions.

Long styled plants selfed yielded 56 Longs; short styled plants selfed with mid pollen yielded 8 Shorts and 1 Long; mid styled plants selfed with long pollen yielded 3 Mids and 1 Long. Shorts crossed with Longs (mid pollen) produced 8 Shorts and 4 Longs; Mids crossed with Longs (short pollen) produced 17 Longs and 8 Mids; Mids crossed with Shorts (long pollen) produced 18 Shorts, 8 Mids, and 14 Longs.

Naturally these results of DARWIN attracted the attention of twentieth century geneticists, and BATESON and GREGORY (1905) were not long in showing that in the Primulas the inheritance of dimorphism may be expressed by the behavior of a single pair of allelomorphs—short style being dominant. Additional experiments with *Primula sinensis* (GREGORY, 1911) showed that there is an association between the factors producing dimorphism and those responsible for the color of the corolla around the tube, which takes the form of a large or a small "eye." A homostyled race with large eye crossed with a short styled race with a small eye yielded F_1 plants having short styles and small eyes. The F_2 generation consisted of 9 short style, small eye: 3 short style, large eye: 3 long style, small eye: 1 homostyle, large eye. Later GREGORY (1915) showed that a short styled plant of *Primula sinensis* behaved as a normal heterozygote when used as a female, but as a homozygote when used as a male. He was not able to determine the reason for this anomalous behavior, however, because the plants used as parents for producing succeeding generations all proved to be homozygous for short style. Still later, GREGORY, WINTON and BATESON (1923) showed that the pair of genes S (short style) and s (long style) are linked with B (blue flowers) and b (slaty flowers), G (green stigmas) and g (colored stigmas), and L (stems and leaf-barks light red) and l (deep red), in that order.

Simple Mendelian results analogous to those discovered for the Primulas were found to hold for Fagopyrum (CORRENS 1921; DAHLGREN 1922), for Pulmonaria (DAHLGREN 1921), and for Linum (UBISCH 1921; CORRENS 1921; LAIBACH 1922, 1923).

Here the subject of dimorphism rested until ERNST, (1925), from experiments on *Primula hortensis* and *Primula hirsuta*, found that two strongly linked gene pairs are concerned. Short style (A) dominates long style (a), and long anthers (B) dominates short anthers (b).

The first post-mendelian work on the inheritance of trimorphism is that of Mrs. BARLOW (1913). The bulk of the data reported in this paper is upon *Oxalis valdiviana*, though a few observations were made upon *Lythrum salicaria*. Mrs. BARLOW found that reciprocal crosses gave like results and therefore that the male and female gametes carry the same factors for flower form, that long styled plants are the ultimate recessives and always breed true to Longs, that mid styled plants give different ratios with the same Short, one yielding no long styled plants and the other yielding an equal number of Mids and Longs. There was indecisive evidence of a second type of short styled plant yielding a ratio approximating 1 Long:1 Mid:8 Short whereas the usual ratio was 1 Long:1 Mid:2 Short. There was also some evidence of a third type of either Mid or Short. In Oxalis, selfed Shorts produced only Shorts; but in Lythrum all three forms appeared in a family of 10 plants coming from such a union.

Mrs. BARLOW published a second paper, dealing primarily with *Lythrum salicaria*, in 1923. In the meantime, Miss VON UBISCH (1921) offered a two-factor interpretation of trimorphism. Plants *aabb* were assumed to be long styled, plants *aaBB* or *aaBb* mid styled, and all plants containing the factor *A* (with or without *B*) short styled. The publication of this paper is an example of a growing practice, not only among geneticists but among other scientists as well, which is of doubtful value. Mrs. BARLOW had used this factorial interpretation as a working hypothesis before the publication of her first paper. This fact she states in her second paper, and I can bear witness to it, for I discussed the matter with BATESON in either 1912 or 1913. It was, of course, the natural hypothesis to use, it being the simplest imaginable. Since there were numerous conflicting data, however, Mrs. BARLOW felt it unwise to suggest the two-factor hypothesis until these objections had been removed. Miss VON UBISCH published the hypothesis nevertheless, *without presenting any experimental work*; and it is now spoken of in the literature as "Ubisch's hypothesis."

Mrs. BARLOW'S data can be discussed to better advantage when our own data are presented.

EXPERIMENTAL WORK

Experiments on the inheritance of trimorphism were started at the BUSSEY INSTITUTION OF HARVARD UNIVERSITY about ten years ago, using certain species of Oxalis as material. Several small populations were raised; but as these species proved to be unsuitable for experimental work, the investigations were soon abandoned. HILDEBRAND obtained all three forms from each of the six legitimate unions while working with Oxalis. We were unable to corroborate his work, our ratios being similar to those found in Lythrum. Generally speaking, Mrs. BARLOW'S results were also similar to those obtained in Lythrum. The only ratio reported as particularly exceptional being one of 6L:102M:101S, where no Longs were to be expected. But we obtained such ratios in Lythrum where the so-called "homozygous" Mids were used. One is inclined to assume, therefore that experimental errors must have crept into HILDEBRAND'S work.

The experiments with *Lythrum salicaria* have been carried on for seven years, both with American material and with material kindly supplied by Mrs. BARLOW in 1920. My thanks are due to Dr. E. S. ANDERSON, Dr. P. C. MANGELSDORF, Dr. R. A. BRINK, Dr. F. G. BRIEGER, and Mr. S. H. YARNELL, who at various times assisted in the work. In addition, I wish to acknowledge my indebtedness to Dr. A. J. MANGELSDORF, who, besides relieving me of much of the drudgery always connected with experiments of this type, helped plan many of the tests.

Self-fertilizations

No selfed seeds from long styled plants were tested. It seemed reasonable to accept as valid the experience of DARWIN and several of his friends and correspondents and of Mrs. BARLOW that Longs selfed with pollen from either the mid stamens or the short stamens give Longs only.

Many efforts were made to self Shorts, but all attempts were unsuccessful. The only data available, therefore, are those of DARWIN and of Mrs. BARLOW. On account of the small numbers involved, all one may say of these results is that selfed Shorts can apparently give either Shorts and Longs, or all three forms. No reliance can be placed on the ratios obtained.

TABLE 1

Results obtained from selfed Shorts.

INVESTIGATOR	UNION	RESULT		
		L	M	S
DARWIN	own m	1	0	8
BARLOW	own $l+m$	1	1	8
BARLOW	own $l+m$	1	1	2
BARLOW	S l	2	0	1

Mids are more easily selfed, as all investigators have found. The results of DARWIN and of Mrs. BARLOW are as follows:

TABLE 2

Results obtained from selfed Mids.

INVESTIGATOR	UNION	RESULT		
		L	M	S
DARWIN	own $l+s$	1	3	0
BARLOW	own $l+s$	0	3	0
BARLOW	own $l+s$	3	9	0
BARLOW	own s, l emas.	1	2	3
BARLOW	own l, s emas.	28	90	0
BARLOW	own l, s not emas.	16	74	1

In 1920, Mrs. Barlow kindly sent me 12 capsules of Mids selfed with their own long pollen. They were from a union that had previously given her 28 Longs and 90 Mids. The plants produced consisted of 47 Longs and 104 Mids, making a total of 75 Longs and 194 Mids from this union,—a noticeable excess of Longs if the ratio to be expected is 1:3. No particular loss of vigor was occasioned by the selfing. The plants, grouped into four classes for vigor, were 9 very low:8 low:26 medium:27 high. Individual capsules yielded the following ratios of Longs to Mids; 13:24, 2:16, 1:4, 11:8, 0:8, 3:10, 4:9, 1:2, 0:7, 0:7, 3:4, and 9:5.

Since selfed Mids, on the two-factor hypothesis, should yield a certain proportion of homozygous Mids, a series of mid styled plants were crossed with pollen from a single presumably "homozygous" Mid (A 19). The selfing in the majority of cases was done with a combination of "long" and "short" pollen; but where the test was made, no difference in result was obtained when either kind was used alone.

A second Mid (D 18) of the same type was also given a thorough test as a male on Mid plants of various origin (table 4). The results were similar to those obtained with Mid A 19.

Table 3

Results from crossing Mids coming from selfed Mids with pollen from a so-called "homozygous" Mid of similar parentage.

CROSS	RESULT		
	L	M	S
B6 ×A19	1	19	0
E6 ×A19	0	5	0
N8 ×A19	0	1	0
A33×A19	0	37	0
A28×A19	0	5	0
A6 ×A19	0	4	0
F15×A19	0	1	0
D8 ×A19	0	12	0
B13×A19	0	3	0
G8 ×A19	0	1	0
N8 ×A19	0	5	0
B14×A19	0	5	0
B18×A19	0	2	0
D14×A19	0	1	0
L8 ×A19	0	3	0
L1 ×A19	0	7	0
A39×A19	2	6	0
Total	3	117	0

Both of these Mids, although they produced ratios totally unlike those produced by ordinary Mids when crossed upon other Mids, do not behave like pure homozygotes. Both produce a certain percentage of "*b*" gametes.

TABLE 4

Results from crossing Mids with pollen from a so-called "homozygous" Mid.

CROSS	ORIGIN, FEMALE	RESULT		
		L	M	S
B3 ×D18	Selfed Mid	0	7	0
B13×D18	" "	0	34	0
C3 ×D18	" "	0	2	0
D12×D18	" "	0	7	0
D16×D18	" "	1	31	0
E11×D18	" "	0	22	0
G15×D18	" "	0	1	0
H35×D18	" "	0	3	0
K10×D18	" "	1	19	0
L5 ×D18	" "	3	7	0
N2 ×D18	" "	0	16	0
N8 ×D18	" "	2	10	0
(A6 ×A19)6 ×D18	Selfed Mid×Mid	0	2	0
(A33×A19)2 ×D18	" " " "	0	20	0
(A33×A19)3 ×D18	" " " "	0	21	0
(A33×A19)19×D18	" " " "	0	10	0
(A33×A19)35×D18	" " " "	0	3	0
(B6 ×A19)7 ×D18	" " " "	1	1	0
(B13×A19)1 ×D18	" " " "	0	20	0
(B13×A19)2 ×D18	" " " "	0	25	0
(B14×A19)4 ×D18	" " " "	0	8	0
(B18×A19)1 ×D18	" " " "	2	15	0
(D14×A19)2 ×D18	" " " "	0	19	0
(L1 ×A19)2 ×D18	" " " "	0	2	0
(L1 ×A19)7 ×D18	" " " "	0	8	0
(N8 ×A19)3 ×D18	" " " "	0	40	0
(LA×MA)8 ×D18	Long×Mid	1	6	0
(ME×LE) 80×D18	Mid×Long	1	14	0
(ME×LE) 87×D18	" " "	1	4	0
(ME×LE) 92×D18	" " "	1	31	0
(N14×D) 18	Selfed Mid	1	27	0

NOTE: The female parent is given first. The figure outside of the parentheses is the number of the plant used from the cross cited.

The ratios obtained in the various families appear to be similar, but owing to the small number of plants appearing in each, the point cannot be determined.

Crosses between Long styled and Mid styled plants

A direct measure of the percentage of "*b*" gametes produced by "homozygous" Mids A 19 and D 18 as males is given by using them on Longs. Long LA crossed with Mid A 19 yielded a ratio of 9 L:85 M:0 S. Long LA crossed with Mid D 18 yielded a ratio of 4L :41 M:0 S. The two results are very similar. Another Long, 1220–5, crossed with Mid D 18, yielded a ratio of 1 L:35 M:1 S. Long LA was also crossed with Mid 1028–3 which should be by origin a "homozygous" descendant of Mid D 18. The ratio was 5 L:30 M:0 S. The total results from these four crosses of Longs by "homozygous" Mids were 19 L:191 M:1 S. The Short, which was one of two produced by a large series of matings between Longs and Mids, can probably be disregarded as the effect of a stray pollen grain. There were 19 Longs out of 210 progeny, thus showing that these "homozygous" Mids produced on the average 9.0 percent "*b*" gametes.

The question arises whether any of the female gametes of these "homozygous" Mids carry the factor "*b*". Four matings are available involving our much used homozygous Mids legitimately crossed with various Longs.

MA 19×LA	produced 1 L:16 M:0 S
MD 18×LA	produced 4 L:64 M:0 S
MD 18×L 1220–2	produced 0 L:14 M:0 S
MD 18×L 1220–5	produced 4 L:27 M:0 S

The two Mids, A 19 and D 18 gave exactly the same results when pollinated with mid pollen from the same Long. From these two crosses, it appears that 6.7 percent of the female gametes of the Mids carry the factor "*b*"; but if all four are averaged, this percentage rises to 6.9.

In addition, a "homozygous" Mid, 1208–21, was crossed with the mid pollen of Long 1202–49, and produced a family with the ratio 1 L:94 M:0 S. Another apparently "homozygous" Mid, 1208–5 crossed with the same Long, 1202–49, produced a family with the ratio 13 L:88 M:0 S. Perhaps the last two Mids regularly produce 1.0 percent and 13.0 percent of "*b*" gametes respectively. On the other hand, these results may be merely extremes that should be averaged with the other crosses. If this is done, the total is 23 L:303 M, or a little over 7.0 percent of "*b*" gametes.

In four crosses Longs were pollinated with pollen from "heterozygous" Mids. The first three were legitimate unions, the last was illegitimate.

LA×MA	produced 13 L:20 M:0 S
LA×M14	produced 31 L:21 M:0 S
LA×M(LA×M14)	produced 1 L: 2 M:0 S
LA×MA	produced 8 L: 3 M:0 S
	Total 53 L:46 M:0 S

Apparently this ratio approaches 1 L:1 M, and the simplest interpretation is to assume that a recessive has been mated with a heterozygous dominant.

Five other crosses were made with "heterozygous" Mids used as females. All were the result of legitimate unions.

M4×L5	produced 7 L: 11 M:0 S
M(4×5)×L(4×5)	produced 54 L: 39 M:0 S
M(der. 4×5)×L(der. 4×5)	produced 58 L: 55 M:0 S
M(der. above cross with S)×LD	produced 28 L: 21 M:0 S
M 1208–4×L 1202–48	produced 62 L: 57 M:1 S
	Total 209 L:183 M:1 S

The single Short appearing in these populations may be assumed to be the result of a stray pollen grain. Disregarding it, "heterozygous Mids" crossed with Longs gave populations totaling 209 Longs and 183 Mids. Again there is an excess of Longs, and this excess is what might be expected if the Mids were producing 46.0 percent "*B*" gametes and 53.0 percent "*b*" gametes.

Crosses between Long styled and Short styled plants

In her 1923 paper, Mrs. Barlow states as one of the objections to the two-factor hypothesis that Longs crossed with Shorts have given only Longs and Shorts, whereas occasional Shorts of constitution *AaBb* should give the ratio 1 L:1 M:2 S when crossed with Longs. Unfortunately, Mrs. Barlow reported only three crosses between Longs and Shorts. Short 14/4×Long yielded a family 19 L:0 M:18 S. Similarly, Short 14/6×Long produced a family 29 L:0 M:22 S. This result is what might have been expected, for the Shorts involved in these crosses probably do not carry Mid. But in the third cross Short 14/5, which had previously given a 1 L:3 M:4 S ratio (37:121:178) when crossed with a Mid, and therefore should be a Short of constitution *AaBb* on the two-factor hypothesis, also gave a ratio of 1 L:1 S (29 L:0 M:27 S) when crossed with a Long.

We made a large number of crosses between Longs and Shorts partly to determine the ratios produced and partly to establish the constitution of various Shorts that were to be crossed with Mids.

Table 5 reports the results from crossing Longs with Shorts which do not carry Mid, by legitimate unions. The first nine crosses listed were duplicated with illegitimate unions. The total results for these particular crosses are 242 L:0 M:245 S, a very good approximation of a 1:1 ratio

TABLE 5

Results from crossing Longs with Shorts which do not carry Mid.
Legitimate unions

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1106	L×S	leg.	A (wild plant)	C (wild plant)	2	0	4
1111	L×S	leg.	A (wild plant)	(SA×MA)3	39	0	43
1113	L×S	leg.	(M×L)4	C (wild plant)	22	0	22
1115	L×S	leg.	(M×L)4	E (wild plant)	19	0	10
1117	L×S	leg.	(M×L)5	B (wild plant)	30	0	11
1126	L×S	leg.	(M×L)63	A (wild plant)	72	0	67
1128	L×S	leg.	(M×L)67	C (wild plant)	22	0	26
1134	L×S	leg.	(M×L)5	C (wild plant)	13	0	12
1203	L×S	leg.	A (wild plant)	((M×L)32×SC)30	33	0	50
1130	L×S	leg.	(M×hom. M, D18)11	(SA×MA)3	6	0	8
1131	L×S	leg.	(M×hom. M, D18)11	(SA×MA)4	2	0	4
1201	L×S	leg.	A (wild plant)	(SA×MA)4	12	0	17
1213	L×S	leg.	A (wild plant)	(M(M×L)32×SE)3	1	0	3
Total for crosses repeated with illegitimate unions					242	0	245
Grand total					263	0	277

of Longs and Shorts. The grand total for these crosses is 263 L:0 M:277 S. The results from duplicates of these nine crosses made by illegitimate unions are reported in table 6. The results are 123 L:0 M:127 S, thus showing that the products of illegitimate and of legitimate unions are similar. (Results of legitimate and of illegitimate crosses are also similar in vigor. Inbreeding reduced vigor, not illegitimate mating.) Adding Cross No. 1105, an illegitimate union not duplicated by a legitimate union, the distribution of the progeny is 128 L:0 M:136 S.

Four crosses were also made by legitimate unions, in which the Shorts were used as the females and the Longs as the males. They are reported in table 7. Cross No. 1215 is the reciprocal of No. 1105 (illeg.). No reciprocals of the other three crosses were made, but some of the crosses in tables 5 and 6 are with parents of similar derivation. The total results are 46 L:0 M:68 S. If this ratio is assumed to be a 1 L:1 S ratio, there is clearly a deficiency of Longs.

TABLE 6

Results from crossing Longs with Shorts which do not carry Mid.
Illegitimate unions

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1107	L×S	illeg.	A (wild plant)	C (wild plant)	5	0	6
1112	L×S	illeg.	A (wild plant)	(SA×MA)3	3	0	1
1114	L×S	illeg.	(M×L)4	C (wild plant)	42	0	48
1116	L×S	illeg.	(M×L)4	E (wild plant)	25	0	21
1118	L×S	illeg.	(M×L)5	B (wild plant)	22	0	27
1127	L×S	illeg.	(M×L)63	A (wild plant)	1	0	3
1129	L×S	illeg.	(M×L)67	C (wild plant)	11	0	13
1135	L×S	illeg.	(M×L)5	C (wild plant)	8	0	3
1203a	L×S	illeg.	A (wild plant)	(M(M×L)32×SC)30	6	0	5
1105	L×S	illeg.	A (wild plant)	A (wild plant)	5	0	9
Total for crosses repeated with legitimate unions					123	0	127
Grand total					128	0	136

Nearly all of the Shorts listed as plants which do not carry Mid were wild plants from the vicinity of Boston (numbered with a letter). From the high frequency of Longs in the places where they were found and from the relative ease of legitimate unions and the relative difficulty of illegitimate unions, it was hardly to be expected that they would carry Mid. The Shorts crossed with Longs reported in table 8, on the other hand, were all derived from crosses with Mids. It was to be expected that many Shorts of such derivation would carry Mid. In table 8, 11

TABLE 7

Results from crossing Shorts which do not carry Mid with Longs.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1215	S×L	leg.	A (wild plant)	A (wild plant)	3	0	4
1223	S×L	leg.	C (wild plant)	(M(M×L)5×SC)6	16	0	24
1224	S×L	leg.	C (wild plant)	(LA×SC)7	11	0	22
1225	S×L	leg.	C (wild plant)	(M(M×L)5×SC)12	16	0	18
Total					46	0	68

TABLE 8

Results from crossing Shorts carrying Mid with Longs.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1109	L×S	leg	A (wild plant)	(SA×MA)2	3	2	5
1110	L×S	illeg.	A (wild plant)	(SA×MA)2	4	1	5
1205	L×S	leg.	A (wild plant)	(M(M×L)32×SC)37	11	16	15
1207	L×S	leg.	A (wild plant)	(M(M×L)32×SC)2	6	4	6
1207a	L×S	illeg.	A (wild plant)	(M(M×L)32×SC)2	1	0	2
1209	L×S	leg.	A (wild plant)	(M(M×L)32×SE)1	3	0	6
1211	L×S	leg.	A (wild plant)	(SA×MA)2	16	16	41
1211a	L×S	illeg.	A (wild plant)	(SA×MA)2	2	0	1
1226	L×S	leg.	A (wild plant)	(M(M×L)32×SC)1	1	3	6
1303	L×S	leg.	(LA×S(SA×MA)2)70	(SA×hom. M(D18))43	5	10	21
1309	L×S	leg.	(LA×S(SA×MA)2)70	(SA×MA)2	1	2	4
1301	S×L	leg.	(SA×hom. M(D18))43	(LA×S(SA×MA)2)70	25	24	76
1306	S×L	leg.	(SA×hom. M(A19))2	(LA×S(SA×MA)2)70	4	51	70
1311	S×L	leg.	(MA×(M(M×L)32×SC)37)5	(MA×(SA×MA)4)48	17	37	37
1312	S×L	leg.	(MA×(M(M×L)32×SC)37)6	(MA×(SA×MA)4)49	5	26	46
Total L×S					53	54	112
Total S×L					51	138	229

such crosses are given but three of them are merely duplicates of other crosses made by illegitimate unions. The ratio exhibited by these populations is 53 L:54 M:112 S, a rather exact 1:1:2 ratio. In nine of these crosses the female parent was the same, the wild Mid plant A. In the other two crosses, the female parent was a Long coming from a cross between a wild Long plant A and a Short known not to carry Mid because of previous tests. The male parents, the Shorts, all but one came from crosses between Mids supposed to be the "heterozygous" type with Shorts known not to carry Mid. The one exception was a Short derived from a cross between a Short known not to carry Mid and a "homozygous" Mid, D18.

There is no reason to believe that the illegitimate unions give results different from those of the legitimate unions. Only 1 Mid was obtained from the three illegitimate unions, it is true; but this result is not extraordinary since the number involved is small (7L:1M:8S).

It may also be noted that one cross, No. 1209, is listed as carrying Mid although no Mids were produced. Only 9 plants make up this population, and it was thought proper to list the family in this table because the Short parent used showed that it carried Mid in Cross No. 1210, reported in table 10.

The crosses where the Shorts were used as females, show even greater variability than when they were used as males. The four populations shown total 51 L:138 M:229 S. This distribution of the three forms can hardly be a 1:1:2 ratio by any stretch of the imagination, especially when one considers that the families are large, that seed production was high and that seed germination was a little better than average, (50–85 percent). Clearly there are two distinct ratios here. Population No. 1301 is a straight reciprocal of population No. 1303. By the two-factor hypothesis the Short parent can only be *AaBb*; therefore it should and does give a possible though bad 1:1:2 ratio (25:24:76). Population No. 1306 is practically a duplicate in origin of population No. 1301, however, and it obviously does not exhibit a 1:1:2 ratio, (4:51:70). In the other two populations, No. 1311 and No. 1312, the Short parents may be either *AaBB* or *AaBb* on the two-factor hypothesis. They arose from crossing "heterozygous" Mids with Shorts that might have carried Mid. In other words, under the two-factor hypothesis the Short parents arose by cross *aaBb*×*AaBb*, and therefore may be either *AaBB* or *AaBb*. The ratios do not have to be of the same type under this supposition, but again we are called upon to explain the "*b*" gametes that "homozygous" Mids appear always to produce.

Crosses between Mid styled and Short styled plants

Mrs. Barlow obtained the largest number of families whose ratios were discordant with those to be expected on the two-factor hypothesis from crosses between Mids and Shorts. She started with a single Short and a single Mid which in reciprocal crosses gave families with the three forms in the ratio 57 L:56 M:139 S. This parental Short crossed again with offspring Mids produced 169 L:195 M:356 S. It is possible to look upon these ratios as the 1:1:2 ratios which should be obtained by crossing Shorts *Aabb* with Mids *aaBb*.

Since some of the Shorts derived from the second group of crosses should be *AaBb*, six chosen at random were crossed back with the original Mid. One produced a family having the distribution 31 L:40 M:87 S,—not a bad 1:1:2 ratio. The other five produced families totaling 87 L: 313 M:382 S,—a possible 1:3:4 ratio. Five shorts carrying Mid to one not carrying Mid where equal numbers are to be expected, appears an unlikely occurrence to Mrs. Barlow; though it is not without the bounds of possibility.

More disquieting for the two-factor hypothesis, as the author notes, are the following cases. A single Short gave a 1:1:2 ratio (28 L:30 M:64 S) with one Mid, and a 1:3:4 ratio (20 L:57 M:63 S) with another Mid. Second, a Short proved not to carry Mid by a cross with a Long, produced 84 L:97 M:137 S when crossed with a heterozygous Mid. Mrs. BARLOW believes that this deficiency of Shorts is serious. More contradictory still is the third case. A Short giving a 1:3:4 ratio (37 L:121 M:178 S) when crossed with a Mid, gave 29 L:0 M:27 S when crossed with a Long. Thus this Short, which should be *AaBb* by the first cross, appears to be *Aabb* by the second.

In our own crosses some similar difficulties with the two-factor hypothesis were found, but they appear to be concerned only with the factor postulated as "*B*." And even where Shorts known not to carry Mid were crossed with Mids, some very fair 1:1:2 ratios were obtained. Nine crosses between Mid females and Short males, and one cross between a Short female and a Mid male, are reported in table 9. Summation of these populations gives the figures 126 L:125 M:243 S.

On the other hand, when it comes to crossing Mids with Shorts which, through crosses with Longs are known to carry Mid, there is practically no evidence for 1:3:4 ratios. The seven crosses between Mid females and Short males, reported in table 10, give populations totaling 22 L:151 M:188 S,—a serious deficiency of Longs. A similar deficiency occurs when Short females are crossed with Mid males. The two crosses made gave a total of 22 L:95 M:139 S. Altogether, in these nine populations

TABLE 9

Results from crossing heterozygous Mids with Shorts known not to carry Mid.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1120	M×S	leg.	M(M×L)32	C (wild plant)	13	16	36
1121	M×S	illeg.	M(M×L)32	C (wild plant)	1	2	2
1124	M×S	illeg.	M(M×L)53	E (wild plant)	18	13	14
1136	M×S	leg.	M(M×L)32	E (wild plant)	17	21	36
1137	M×S	leg.	M(M×L)32	E (wild plant)	2	3	6
1202	M×S	leg.	A (wild plant)	(SA×MA)4	28	31	48
1204	M×S	leg.	A (wild plant)	(M(M×L)32×SC)30	3	3	17
1214	M×S	leg.	A (wild plant)	(M(M×L)32×SE)3	17	8	35
1227	M×S	leg.	A (wild plant)	(SA×MA)3	3	7	5
1216	S×M	leg.	A (wild plant)	A (wild plant)	24	21	44
Total					126	125	243

there were 44 L:246 M:327 S. Only one family, as may be seen by reference to the table, gave a fair 1:3:4 ratio. Cross No. 1302 yielded 20 L:54 M:71 S. The remaining families show a great deficiency of Longs and an excess of Mids over what is demanded by the two-factor hypothesis.

Table 10

Results from crossing heterozygous Mids with Shorts known to carry Mid.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1206	M×S	leg.	A (wild plant)	(M(M×L)32×SC)37	0	12	15
1208	M×S	leg.	A (wild plant)	(M(M×L)32×SC)2	3	17	13
1212	M×S	leg.	A (wild plant)	(SA×MA)2	3	24	21
1210*	M×S	leg.	A (wild plant)	(M(M×L)32×SE)1	7	37	40
1308	M×S	leg.	(hom. M(D18)×LA)17	(SA×MA)2	4	7	12
1321	M×S	leg.	(hom. M(D18)×LA)17	(M(M×L)32×SC)37	3	8	21
1304	M×S	leg.	(hom. M(D18)×LA)12	(SA×hom. M(A19)2	2	46	66
1305	S×M	leg.	(SA×hom. M(A19))2	(hom. M(D18)×LA)12	2	41	68
1302	S×M	leg.	(SA×hom. M(D18))43	(hom. M(D18)×LA)17	20	54	71
Total					44	246	327

* Short not *known* to carry Mid.

The only other bit of evidence available is shown in table 11, where the results from crossing Shorts known not to carry Mid with "homozygous" Mids are listed. The two populations have a total of 5 L:36 M:45 S.

Table 11

Results from crossing Shorts known not to carry Mid with "homozygous" Mids.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1217	S×M	leg.	A (wild plant)	hom. M(D18)	4	30	37
1218	S×M	leg.	A (wild plant)	hom. M(A19)	1	6	8
Total					5	36	45

According to the two-factor hypothesis, 1 M:1 S should be the result. Again, the supposedly "homozygous" Mids, D18 and A19, are shown to be producing in the neighborhood of 10 percent "*b*" gametes.

DISCUSSION

My interest in heterostyly was aroused originally by its apparent relationship to self-sterility, a subject upon which I have been working more years than I like to recall. In *Lythrum salicaria*, short styled plants are almost completely self-sterile. DARWIN and BARLOW have raised 26 plants from selfed Shorts. I have obtained none,—no seeds being formed even on vigorous isolated plants. Selfed Longs produce seeds more frequently; selfed Mids are the most prolific of all. Yet even so, the species may be classed as a self-sterile species. It seemed quite probable, therefore, that the behavior of trimorphic plants would be interpretable as self-sterility linked in some manner to morphological differentiation of the flowers.

Unfortunately the genetic interpretation of self-sterility based upon selective pollen tube growth that has been found adequate for certain species of Nicotiana, Antirrhinum and Veronica does not fit this case. There is differential pollen-tube growth, as will be shown in a later paper; but there is no evidence, either of intra-sterile groups of plants among those having the same flower form or of genetic factors controlling self-sterility linked with flower-form differences, that is at all comparable with the situation in Nicotiana, Antirrhinum and Veronica. Mids and Shorts have two kinds of pollen differing in color, size and in nutrient content (starch or fat) and even Longs have two different sizes of pollen; nevertheless the two kinds of pollen of a particular plant bear the same genetic factors for heterostyly,—a matter proved by the identical results of legitimate and illegitimate unions. None of the ordinary tests useful in proving the genetic behavior of ordinary self-sterile plants, in fact, give us information on the genetics of self-sterility in Lythrum. A physiological interpretation may be found which will fit both types, but a genetic interpretation is unlikely.

The genetic interpretation used as a working hypothesis during the earlier part of these experiments,—the two-factor scheme by which Longs are represented by the formula $aabb$, Mids by the formulæ $aaBb$ and $aaBB$ and Shorts by factor A epistatic to B,—comes just near enough to the facts to make comparisons interesting. The evidence for the case is summarized in table 12. One can hardly examine this evidence without feeling that the two-factor hypothesis is an approach to the truth. Yet it is not the whole truth. There are discrepancies that demand another interpretation. The Long is evidently the ultimate recessive. A single epistatic factor apparently accounts for the Short. But wherever the factor or factors responsible for the Mid condition occur, results are irregular. Selfed Mids produce more Longs than are needed for a ratio of 1L:3M. Presumably "homozygous" Mids throw Longs, that is, they always produce the recessive gametes, even in crosses with Shorts.

Again, there is a marked deficiency of Longs, and in some cases an excess of Shorts, in crosses which, from the derivation of the parents, ought to be *AaBb*×*aaBb* or *aaBb*×*AaBb*. There is also the question of whether or not the elimination of gametes and zygotes commonly found has a genetic significance. The six kinds of pollen found in the three forms exhibit from 10 to 30 percent of abortive grains. This fact may not have a bearing on the genetic interpretation of heterostyly since the distribution of percentages of abortive pollen within each type shows high variability. The occurrence of non-viable zygotes seems more important. Counts of seeds in hundreds of capsules, taken together with germination tests, *show that the same plants used as females regularly eliminate more zygotes in some crosses than in others.*

TABLE 12

Summary of tests of the two-factor interpretation of trimorphism in Lythrum salicaria.

TEST	RESULT EXPECTED	RESULT OBTAINED
Selfed Longs (*aabb*)	All Longs	Satisfactory
Selfed Mids (*aaBb*)	1L:3M	Excess Longs
Mids (*aaBB*)×Mids (*aaBb*)	All Mids	Unsatisfactory. Longs produced
Selfed Shorts (*Aabb*)	Longs and Shorts	Satisfactory. No good ratio
Selfed Shorts (*AaBb*)	All three forms	Satisfactory. No good ratio
Longs (*aabb*)×Mids (*aaBb*)	1L:1M	Satisfactory. Excess Longs
Mids (*aaBb*)×Longs (*aabb*)	1L:1M	Satisfactory. Excess Longs
Longs (*aabb*)×Mids (*aaBB*)	All Mids	Unsatisfactory. Longs produced
Mids (*aaBB*)×Longs (*aabb*)	All Mids	Unsatisfactory. Longs produced
Longs (*aabb*)×Shorts (*Aabb*)	1L:1S	Satisfactory
Shorts (*Aabb*)×Longs (*aabb*)	1L:1S	Unsatisfactory. Excess Shorts
Longs (*aabb*)×Shorts (*AaBb*)	1L:1M:2S	Satisfactory
Shorts (*AaBb*)×Longs (*aabb*)	1L:1M:2S	Unsatisfactory. Deficiency Longs
Mids (*aaBb*)×Shorts (*Aabb*)	1L:1M:2S	Satisfactory
Shorts (*Aabb*)×Mids (*aaBb*)	1L:1M:2S	Satisfactory
Mids (*aaBb*)×Shorts (*AaBb*)	1L:3M:4S	Unsatisfactory
Shorts (*AaBb*)×Mids (*aaBb*)	1L:3M:4S	Unsatisfactory. Variable ratios
Shorts (*Aabb*)×Mids (*aaBB*)	1M:1S	Unsatisfactory. Longs produced

Not having the seed count and the germination data segregated according to type of cross, I very stupidly overlooked the obvious import of these facts. The common Mendelian schemes were tried,—additional factors, linkage, multiple allelomorphs; but such hypotheses raised up more obstacles than they cleared away. Cytological examinations of all three forms were made, and TISCHLER'S work (1917, 1918), showing 24 pairs of chromosomes for each type with no abnormal behavior, was confirmed. Thus there was no good ground for assuming non-disjunction or unequal crossing over, even if such assumptions could have been fitted to the facts. But the obvious interpretation presented by the germination tests, balanced lethals, was overlooked until Dr. P. W. WHITING suggested that I try such a scheme.

Retaining the idea that the Long is the ultimate recessive and that the Short is conditioned by an epistatic factor "A," let us assume that the Mid is conditioned by duplicate linked factors M_a and M_b that are lethal in the homozygous condition. Owing to the various possibilities of mating, four kinds of Mids can exist, namely (1) $\frac{M_aM_b}{m_am_b}$, (2) $\frac{M_am_b}{m_aM_b}$, (3) $\frac{M_am_b}{m_am_b}$ and (4) $\frac{m_aM_b}{m_am_b}$. The first two types probably ought to be more frequent in Nature than the last two.

For several reasons, among which is the probability that Mids of the constitution $\frac{M_aM_b}{m_am_b}$ are more easily selfed, we may assume that the population produced by our selfed Mids came from a mating of this type. Solving the proper algebraical equation for a crossover percentage that will fit the ratio of 75 L:194 M obtained by this union, we get 10 percent. A 10 percent crossover value will give us 28.8 L:71.2 M. Mids of constitution $\frac{M_aM_b}{m_am_b}$ will form 57.7 percent of the progeny, those of constitution $\frac{M_am_b}{m_am_b}$ and $\frac{m_aM_b}{m_am_b}$ will form 12.8 percent of the progeny, while those of constitution $\frac{M_am_b}{m_aM_b}$ will form 0.71 percent of the progeny. Mids of constitution $\frac{M_am_b}{m_am_b}$ or $\frac{m_aM_b}{m_am_b}$ selfed will give 1 L:2 M, like the yellow mice. Mids of the constitution $\frac{M_am_b}{m_aM_b}$ selfed will give L 0.5:M 99.5, the Mids produced being 80.5 percent of type 2, 9 percent each of types 3 and 4 and 1 percent of type 1. This type of Mid, $\frac{M_am_b}{m_aM_b}$, is our supposedly "homozygous" Mid such as is found in plants D18 and A19.

We may take it then that the crosses shown in table 3 and 4 are crosses in which the females are partly $\frac{M_aM_b}{m_am_b}$ (Cross 1), partly $\frac{M_am_b}{m_aM_b}$ (Cross 2), while the males are $\frac{M_am_b}{m_aM_b}$. Cross 1 yields 4.3 percent Longs; Cross 2, like the selfing of Mid of Type 2, yields 0.5 percent Longs. It is possible, though not highly probable from their derivation, that some of these crosses also involved females of constitution $\frac{M_a m_b}{m_a m_b}$ and $\frac{m_a M_b}{m_a m_b}$. If so, the results would not be changed substantially, as these types mated with $\frac{M_a m_b}{m_a M_b}$ yield 3.3 per cent Longs. In the first series the result was 3L:117M: In the second series the total was 15 L:507 M. The result was just a little under 3.0 percent Longs, therefore, in each case.

Considering now the crosses between Mids and Longs, we have four possibilities. The common type of "heterozygous" Mid is naturally $\frac{M_a M_b}{m_a m_b}$. This type, when crossed with Long (continuing to use the 10 percent crossover value), yields 45 percent L:55 percent M. In our experiments we obtained 53.3 percent L:46.7 percent M. This result is hardly a good fit, but not sufficiently anomalous to negate the hypothesis, I think. The other two types of "heterozygous" Mid, the rarer types, should give 1 L:1 M when crossed with Longs. If any such plants should have been present in the tests, it would tend to lessen the slight departure from the expected ratio.

The so-called "homozygous" Mids, $\frac{M_a m_b}{m_a M_b}$, crossed with Longs give 5 percent L:95 percent M. We obtained 8 percent Longs in a total of 636 plants. Leaving out one extreme family which produced 13 L to 88 M, the ratio was 7 percent Longs.

The present assumptions make no change in the expected ratio for crosses between Longs and Shorts which do not carry Mid. The ratio should be 1 L:1 S, as was found in the crosses reported in tables 5, 6 and 7. There is a slight excess of Shorts (46 L:68 S) in table 7, but no more than would be expected rather frequently in families of 114.

So also the presumably rarer types of Short, $\frac{A}{a}\ \frac{M_a m_b}{m_a m_b}$ and $\frac{A}{a}\ \frac{m_a M_b}{m_a m_b}$, will give 1 L:1 M:2 S ratios when crossed with Longs, just as in the simple two-factor hypothesis. The common "heterozygous" Short, $\frac{A}{a}\ \frac{M_a M_b}{m_a m_b}$, crossed with Long, should give a ratio of 22.5L :27.5M: 50S. The so-called "homozygous" Short, $\frac{A}{a}\ \frac{M_a m_b}{m_a M_b}$, crossed with Long should give a ratio of 2.5 L:47.5 M:50 S.

Now if we examine table 8, where such crosses are listed, we see that there are two distinct ratios. Totaling the figures of all the crosses except No. 1306 and 1312, gives 95 L:115 M:225 S. This is a percentage ratio of 21.8 L:26.4 M:51.8 S, which is a very satisfactory fit for the first ratio. The other two crosses yielded 9 L:77 M:116 S, a percentage ratio of 4.4 L:38.1 M:57.5 S, only a slight deficiency of Mids from expectation for the second ratio.

Crosses 1301 and 1311, presumably from Shorts carrying "heterozygous" Mid and Crosses 1306 and 1312, presumably from Shorts carrying "homozygous" Mid, are to be regarded as critical tests. From their derivation, they should, on the two-factor hypothesis, give similar results; on the balanced-lethal interpretation they may give different results because of crossovers. On the two-factor hypothesis, for example, "homozygous" Mids A19 and D18 should be *aaBB* and crossed with the same Short should give only Shorts *AaBb*; on the "balanced-lethal" interpretation the same cross may give Shorts $\frac{A}{a}\ \frac{M_aM_b}{m_am_b}$ and $\frac{A}{a}\ \frac{M_am_b}{m_aM_b}$.

A great many kinds of matings are possible between Mids and Shorts. We may neglect those where either the Mids or the Shorts contain the formula $\frac{M_am_b}{m_am_b}$ or $\frac{m_aM_b}{m_am_b}$. In the first place they would give results approaching those of the two-factor hypothesis and of certain other cases calculated on the balanced-lethal hypothesis; in the second place, plants of these constitutions should be rare from theoretical considerations and are actually rare as shown by the analysis of our results. For example, a Mid of this type crossed with a Short not carrying Mid gives a 25 L: 25 M:50 S ratio, crossed with a "heterozygous" Short gives a 15 L: 35 M:50 S ratio, crossed with a "homozygous" Short gives a 1.7 L: 48.3 M:50 S ratio, and crossed with a short like itself except for the additional factor A gives a 16.7 L:33.3 M:50 S ratio.

We are interested primarily in the ratios expected when "heterozygous" and "homozygous" Mids are crossed with the three common types of Short.

"Heterozygous" Mids, $\frac{a}{a}\ \frac{M_aM_b}{m_am_b}$, crossed with Shorts $\frac{A}{a}\ \frac{m_a\,m_b}{m_am_b}$, the Shorts which do not carry Mid, give a 22.5 L:27.5 M:50 S ratio. The same Mids crossed with "heterozygous" Shorts, $\frac{A}{a}\ \frac{M_aM_b}{m_am_b}$, give a 14.4L: 35.6M:50S ratio. When crossed with "homozygous" Shorts, $\frac{A}{a}\ \frac{M_am_b}{m_aM_b}$, these Mids give a ratio of 2.2 L:47.8 M:50 S.

"Homozygous" Mids, $\frac{a}{a}\,\frac{M_a m_b}{m_a M_b}$, crossed with Shorts which do not carry Mid give a ratio of 2.5 L:47.5 M:50 S. When crossed with "heterozygous" Shorts, these Mids give a ratio of 2.2 L:47.8 M:50 S. And finally, when these Mids are crossed with "homozygous" Shorts, they give a ratio of 0.25 L:49.75 M:50 S.

Any Mids other than the so-called "homozygous" Mids when crossed with Shorts known not to carry Mid, yield very nearly a 1 L:1 M:2 S ratio; and this is what was obtained as seen in table 9.

The two crosses shown in table 11 are between Shorts known not to carry Mid and "homozygous" Mids. Theoretically the ratio is 2.5 L:47.5 M:50 S; actually it is 5.8 L:41.9 M:52.3 S.

The crosses shown in table 10 are not so easy to analyze. We know, however, that the Mid used in the first four crosses is a "heterozygous" Mid, and that the Shorts must be either "heterozygous" or "homozygous." In the first case the ratio should be 14.4 L:35.6 M:50 S; in the second case the ratio should be 2.2 L:47.8 M:50 S. Actually the plants obtained were 13 L:90 M:89 S. The fit is not bad if one assumes that both types of Short were present.

In the next five crosses both the Mids and the Shorts may be either "heterozygous" or "homozygous," *even if two or more are derived from a single source cross.* Little can be said for the ratio exhibited by the two families No. 1308 and No. 1321, giving together the figures 7 L:15 M:33 S. The number of plants is too small. The last three crosses shown in the table form the critical test. Crosses No. 1304 and No. 1305 are reciprocals. Together they gave 4 L:87 M:134 S, a ratio of 1.8 L:38.7 M:59.5 S, which is a reasonable expectancy for a cross in which *either* the Mid or the Short parent is "heterozygous" and the other parent "homozygous." In Cross No. 1302, both parents were derived from the same matings as in the reciprocal cross just described, and in such a way that by the simple two-factor hypothesis the crosses ought to be duplicates of each other. Yet Cross No. 1302 gives quite a different result. The figures are 20 L:54 M:71 S, a ratio of 13.8 L:37.2 M:49.0 S. This is a very good fit for a cross between a "heterozygous" Mid and a "heterozygous" Short. And it is a result to be expected on the balanced-lethal hypothesis from parents of the same derivation that gave the results obtained in Crosses No. 1304 and No. 1305. Under the two-factor hypothesis the discordance is outstanding and inexplicable.

In all the tests therefore, the balanced-lethal hypothesis fits the facts if one allows for the fluctuations due to random sampling and for the obvious roughness of the crossover determination.

SUMMARY

In this paper it is shown that trimorphism in *Lythrum salicaria* is due to three factors. The long styled plant is the ultimate recessive. The mid styled plant is due to duplicate factors in the same linkage group having a crossover value of about 10 percent. These factors are lethal when in the homozygous condition. The mids most commonly found have the constitution $\frac{M_a M_b}{m_a\ m_b}$. Mids $\frac{M_a m_b}{m_a\ m_b}$ were also tested in numerous combinations. The short styled plants are conditioned by an independent factor A. They may or may not carry the factors that condition Mid.

There is some evidence that crossing over is slightly less in the female than it is in the male.

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GENETICS, SEPTEMBER, 1927

TABLE OF CONTENTS

PAGE

EAST, E. M., The inheritance of heterostyly in *Lythrum salicaria* . . 393

CARVER, W. A., A genetic study of certain chlorophyll deficiencies in maize 415

OWEN, F. V., Inheritance studies in soybeans. I. Cotyledon color . . 441

LESLEY, MARGARET MANN, AND FROST, HOWARD B., Mendelian inheritance of chromosome shape in Matthiola 449

[This number of GENETICS (12:393–460) was issued Aug. 25, 1927]

THE GROWTH AND DEVELOPMENT OF PLASTIDS IN *LUNULARIA VULGARIS, ELODEA CANADENSIS,* AND *ZEA MAYS*

CONWAY ZIRKLE

Reprinted from AMERICAN JOURNAL OF BOTANY, Vol. XIV., No. 8, 429–445, October, 1927

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(Received for publication January 3, 1927)

In certain Conjugatae, Chlorophyceae and Archegoniatae the chloroplasts pass through the entire life cycle relatively unchanged. They are reproduced exclusively by the division of pre-existing plastids and are apparently permanent cell organs, in many ways comparable with the nucleus. In the higher plants, however, the life history of the chloroplasts is quite different and much more complicated. In certain stages of the life cycle no chloroplasts occur. When they reappear it is evident that they must either have originated *de novo* or have passed through the preceding stages in a much less conspicuous form. Many investigators have reported the existence of small, relatively undifferentiated bodies in the meristematic tissue, which gradually develop into chloroplasts. It is the object of the present investigation to record certain of the significant changes which occur in these bodies during the course of their development.

Any investigation of the growth and development of plastids must deal to some extent with the problem as to whether or not the plastids are derived from mitochondria. The numerous papers bearing on this question have been very adequately summarized by Cavers (1) and Cowdry (3) and more recently by Emberger (6) and Guilliermond (9), so no review of the literature will be essayed here. The divergent views of various investigators have given rise to a considerable controversy. There is at present a great deal of uncertainty as to the nature and function of mitochondria[1] and it is highly probable that diverse cell elements have been included under that term by different investigators. In the present investigation, developing plastids were frequently traced back to primordia which, when fixed and stained, were morphologically indistinguishable from mitochondria. No general conclusions as to the relationship of plastids and mitochondria could be drawn from these instances, however, for it seems certain, as Kingery (12) has suggested, that the form the plastid primordia take depends to a great extent upon the fixative used.

As fixation alters the finer structure of the plastid so markedly (Zirkle, 23), it is desirable to investigate the plastid development in living cells. In the present investigation this was done whenever possible. It was necessary, however, in order to examine certain meristematic cells, to section the material. Both pith sections and frozen sections were cut and mounted in a .4 *M* solution of sucrose. The plastids they contained were then compared with those in unaltered cells. No change in the gross microscopic structure of the plastids could be observed. Both living cells and sections thus prepared were stained with various vital stains, neutral red, methylene blue, Janus green B, etc., but the use of vital stains was found, as had been reported by Randolph (20), to be of little aid in distinguishing either the growing plastid or plastid primordia.

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[1] In the present paper the term *mitochondria* means any thread-like or rod-like cytoplasmic inclusion appearing in cells fixed with bichromate and not appearing in those fixed with acetic acid fixatives.

It was a relatively easy matter to trace back the development of the plastid to a small colorless primordium, either globular or slightly prolate-spheroidal in shape. These primordia, having almost the same index of refraction as the surrounding cytoplasm, were very difficult to observe, and it was found impossible to distinguish them in the living cells from other cytoplasmic inclusions. As they were so near the border line of visibility it was not possible to determine whether or not they reproduced only by division or were being continually formed *de novo* (Randolph, 20). The difficulty was not due primarily to the minute size of the primordia but rather to their low visibility even when the sections were stained. It was only in fixed sections cleared and mounted in balsam that any crucial evidence could be obtained. Search was made for a fixing agent which would preserve the primordia unaltered in their microscopic structures, through the dehydration and clearing process, and which would so mordant them that they could be colored with the common histological stains. Various fixatives were tried and the fixed and stained primordia were compared as far as possible with those living. No entirely satisfactory fixative was found. It was possible, however, to demonstrate the presence of self-perpetuating bodies in the apical cell of Lunularia and the primary meristem of Elodea and Zea which developed into plastids in the older tissue.

In brief, the usual nuclear fixatives which contain acetic acid (Flemming's, Bouin's, Carnoy's, Gilson's, etc.) were worthless for this purpose; for, while they did not alter the size or the shape of the mature plastid, they either dissolved out the primordia or allowed them to be dissolved by the dehydrating alcohol. The usual mitochondrial fixatives (such as Altman's, Benda's, Rigaud's, and Champy's) were also used, as were various combinations of potassium bichromate, osmic acid, corrosive sublimate, and formalin. Mottier's modification of Flemming's solution gave by far the best fixation of the mature plastids, but it so disorganized the dense cytoplasm of the growing point that the primordia could not be recognized. Osmic acid, used alone or mixed with bichromate as in Altman's, fixed the true fats and certain mixtures of cholesterin and unsaturated fatty acids (Cramer, 4). Not only was the cytoplasm of the meristem thus fixed so darkened as to obscure any observation of the inclusions but it also contained many granules other than plastid primordia. There were also certain disadvantages in using fixatives containing potassium bichromate. These did not alter the size and shape of the mature plastids but greatly enlarged those pores in the stroma connecting the central vacuole of the plastid with the surrounding cytoplasm. Frequently the plastid thus fixed appeared as a darkly staining meshwork surrounding a starch grain (Pl. LI, fig. 3; Pl. LIV, fig. 18). Sometimes these fixatives caused the plastid to shrink to one side of a single large starch grain (Pl. LII, fig. 9). Primordia which in the living cell were globular or prolate-spheroidal became rod-shaped when thus fixed. As these fixatives did not penetrate particularly well, the material had to be cut into small pieces before fixation. In Zea it was necessary to split open the plumule sheath if the stem growing point was to be properly fixed before its cytoplasm became disorganized. In spite of these disadvantages the bichromate fixatives were the ones primarily used. The alterations they brought about in the plastid structure could be recognized and proper correction made. They so mordanted the tissue that only the immature plastids, mitochondria, nucleolus and granules of protein reserve food were deeply stained with Heidenhain's haematoxylin. Fats and oils thus fixed and stained appeared as yellow globules quite distinct from the dark blue rod-shaped plastid primordia. The most satisfactory combination tried was that of Erliki, potassium bichromate 2.5 percent and copper sulphate 1 percent. This mixture penetrated and hardened the tissue much better than bichromate alone. If the material to be fixed had a waxy coating or if it contained resin ducts or much stored oil, a green precipitate would form after a prolonged fixation. This, however, in no way interfered with the fixation of the primary meristem. The addition of .5 percent urea aided penetration but did not appreciably improve the fixation. Thirty-six hours in the fixative was quite sufficient.

LUNULARIA VULGARIS

It is well known that the structure of the thallose liverworts is very easily altered by environmental changes. They are especially susceptible to alterations in humidity and when grown in a saturated atmosphere are often changed beyond recognition. The development of plastids in *Lunularia vulgaris* is not only conditioned indirectly by the environment acting upon the plant as a whole but also by a direct action of the environment upon the plastid, as in the case of light. The growth and development of the plastid is not rigidly bound up with the growth of the plant; for in a rapidly growing thallus, cells a given distance from the apical cell will contain immature plastids, though in a slowly growing thallus their plastids would be mature. As the apical cell of Lunularia is deep within the thallus, it requires fewer cell divisions in younger, thinner thalli than in the older ones which have acquired their maximal thickness to bring the plastids to the dorsal surface and hence to the light supply. In the present investigation, the size and form of the plastid has been studied with relation to its position in the thallus, *i.e.*, its position relative to the apical cell. The relationship found may hold only with plants grown under conditions similar to those used here. The development of the plastid should not be changed essentially in plants grown under other conditions, but the various stages might occur either nearer to or farther from the apical cell. The thalli investigated were mature ones grown exposed to light in a greenhouse but protected from direct sunlight by other plants. The soil was damp and the air moist but by no means saturated.

Longitudinal sections were made through the apical cell perpendicular to the thallus. Plastid development was traced from this cell (1) to the mature epidermal cells, (2) through the subepidermal cells to the palisade cells and (3) to the cells deep within the thallus.

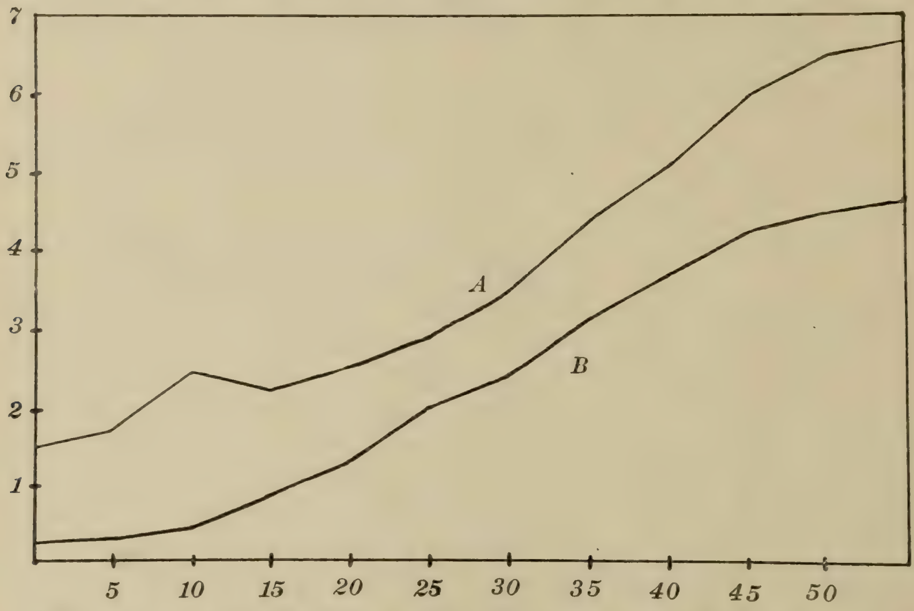
In the apical cell, fixed with Erliki's solution, are numerous rod-shaped primordia about 1.5 μ long. Sometimes two or more are united end-to-end (Pl. LI, fig. 1); at times they form chains. These chains occur too regularly to be accounted for as accidental grouping of the primordia by the cytoplasmic current. The occurrence of the plastid primordia in chains and never in plates would indicate that they divide regularly in one plane. Occasionally chains will have the form of a "Y" (Pl. LVI, fig. 23) which would indicate that occasionally a primordium divides in a second plane. The division of plastid primordia in the apical cell of a kindred form has been reported (Mottier, 18). There is also evidence that these primordia do not originate *de novo*, since in a single cell they are all approximately the same size and never grade off into smaller bodies on the border-line of visibility, as would be expected if they were being continually formed anew.

The plastid primordia are variously located within the cell. Sometimes they are scattered throughout the cytoplasm and sometimes they are grouped about the nucleus. Prior to cell division they collect into two groups on opposite sides of the nucleus, and when division occurs each group goes into one of the daughter cells. There does not seem to be any constant number within a cell and there was no evidence that exactly half of the primordia went to the daughter cells. This grouping of primordia in Zea is shown in Plate LIV, figure 15.

In living apical cells and in frozen sections the primordia are quite refractive and it is difficult to determine whether or not they contain any pigment. They appear yellowish and even in this stage may be colored by carotin and xanthophyll. This cannot be stated with certainty. Attempts to stain these primordia with vital stains gave very irregular results. They were not stained by weak solutions of Janus green B which Cowdry (2) found to be highly specific for mitochondria. The cytoplasm of the mucilage-secreting cells between the ventral scales which surround the growing point absorbed this stain readily and seemed to be full of mitochondria. When germinating gemmae are placed in such a stain and examined under the low power of the microscope they appear to have deeply stained apical cells. When the gemmae are teased apart, however, it can be seen that the deeply stained cell is not the apical cell but one which curves around the apex and secretes mucilage. The light yellowish-green color of primordia in the meristematic cells immediately surrounding the apical cell indicates that here they contain not only the usual yellow pigments but also some chlorophyll.

In addition to the plastid primordia, the fixed and stained apical cell contains several globular bodies of varying size but averaging .33 μ in diameter. These are the oil droplets, probably reserve food, which have been rendered insoluble by the bichromate.

In longitudinal sections through the growing point, plastids can be seen in various stages of development, ranging from mature ones in the main photosynthetic tissue of the thallus, the palisade cells, to the primordia in the apical cell. A convenient way to trace this development is to record the structure of the plastids and changes which occur in them in cells at various distances from the apex. The cell lineage itself is easy to trace, and as we proceed from the apical cell the plastids become regularly more mature. For about five cells from the apical cell the lineage is along the surface (Pl. LI, fig. 1). The primordia have increased slightly in size but appear otherwise unchanged. As has been stated, in living cells they are ellipsoids and light green in color. The line of development at about this point becomes subepidermal. The plastid primordia continue to increase slowly in size and at ten cells from the apical cell they become, on the average, 2.5 μ by .5 μ, but are otherwise unchanged. It is interesting to note here that these primordia are completely disorganized by the "nuclear" fixatives but when so fixed the cytoplasm of these cells stains much more darkly than that in any other cells of the thallus. At approximately fifteen cells from the apex the primordia undergo several fundamental changes. The cells are now immediately below the upper surface of the thallus (fig. 1). In sections of living material it can be seen that the plastids are no longer a pale yellowish green but are a deep grass green, the same shade as the mature chloroplasts. They are no longer soluble in fixatives containing acetic acid and their size and shape are well preserved by the fixatives of Flemming, Bouin, Gilson, etc. In material fixed with Erliki's solution they appear to have become suddenly thicker and shorter, now measuring on an average 2.25 μ × 1 μ (text fig. 1). On checking these measurements with plastids in living material it develops that this sudden change of shape is apparent and not real. At this point the plastids have reached a stage of their development where they are no longer made rod-shaped by the fixative but retain their true shapes (Pl. LI, fig. 1). At twenty-five cells from the apex the plastids average 4 μ × 2 μ and it is here that small starch inclusions first appear. There is no reason, however, for believing that the starch inclusions must always occur at this fixed point in plants grown under conditions other than those here used. Cell division ceases about thirty-five cells back from the apex. Here the plastids average 4.5 μ × 3 μ, and when fixed with a bichromate fixative first show the enlarged pores which lead from their central cavities to the surrounding cytoplasm (Pl. LI, fig. 3). Fixed with Mottier's modification of Flemming's solution, they appear as in figure 4. No marked change occurs from here on, though in the older thallus some few plastids will reach a size of 7 μ × 5 μ (text fig. 1).



Text Fig. 1. Average length (*A*) and breadth (*B*) of developing plastids in the line of cells extending from the apex to the palisade cells of Lunularia. Ordinates are dimensions of the plastids; abscissae are the distances, in number of cells, from the apical cell.

Plastid development in the epidermal cells parallels the development of plastids in the sub-epidermal cells for about the first twenty-five cells. They then no longer increase in size, but appear to stop dividing sometime before the cells cease to divide, since they become less numerous in each cell. Other cytoplasmic inclusions in these cells are of interest. These are globules ranging in size from a diameter of 3 μ on down to the limits of visibility (Pl. LI, fig. 1). Their distribution covers a range of about thirty cells extending backward from the apical one, and they are especially concentrated in the region from ten to twenty cells from the apex. When fixed with osmic acid alone or in combination with bichromate they stain black, become irregular in outline, and are difficult, especially when small, to distinguish from the plastid primordia. When they are fixed with Erliki's solution and stained with iron-alum haematoxylin they appear yellow and can easily be distinguished from the blue-staining plastid primordia. These globules seem to be merely oil droplets rendered insoluble by the bichromate. They probably are reserve food.

The various stages in the development of the plastids in the cortical cells cannot easily be traced for they reach their full size, 4 μ × 3 μ, almost immediately behind the apical cell. They are not as dark a green as those in the palisade cells, and nearly always contain a single starch grain which fills their central cavities. When fixed with bichromate fixative their pores become enlarged. This enlargement of pores also occurs when they are fixed with Mottier's modification of Flemming's solution, which is quite different from the reaction of the plastids in the palisade cells (Pl. LI, fig. 4).

Elodea canadensis

In longitudinal sections of the apical bud of Elodea all stages of plastid development can be observed, from the mature plastids in the enclosing leaves to the minute primordia of the primary meristem. The primordia occur in all of the meristematic cells and are considerably smaller than those in the apical cell of Lunularia. When fixed with Erliki's solution they appear as minute rods .5 μ × .25 μ (Pl. LIII, fig. 10). In living cells these primordia are prolate-spheroidal in shape and contain no pigment whatever. They can be identified only with great difficulty. Indeed, they remain colorless in the rudimentary leaves and axillary scales and even in the bases of many mature leaves as long as these are shaded by other leaves. In Elodea the plastid primordia seem much more simplified than in Lunularia, and they remain colorless much longer. They do not increase noticeably in size or complexity throughout the entire meristematic region. It is perhaps suggestive that the development of the plastid primordia should not take place in a region of rapid cell division. It would seem to aid the economy of the cell for the primordia to remain small and to divide rapidly in a rapidly growing region. This point will be discussed later in the treatment of the root tips of Zea. The division of primordia, as in Lunularia, is in one plane only.

Beginning with the dermatogen, the primordia can be traced to the rudiments of the axillary scales and leaves and to the epidermis of the stem. In the axillary scales the further development of the primordia into plastids is inhibited. No axillary scale was observed to become green even when the bases of the leaves adjoining it on both sides were distinctly colored. The cytoplasm in the cells of the scale is much denser than in the cells of the stem and the leaves adjoining. When fixed with acetic acid fixatives it does not appear to be as disorganized as cytoplasm usually is. The plastid primordia in these scales elongate but do not divide, and develop into bodies which when fixed with a bichromate fixative are microscopically identical with mitochondria (Pl. LI, fig. 2). Such bodies have been reported and figured by Lewitsky (16). The general appearance of these scales strongly suggests that their function is one of mucilage secretion. The relationship of plastid primordia to mitochondria-like bodies will be discussed later.

The development of the plastid primordia in the small, tightly packed leaves about the stem apex consists of a gradual and regular increase in size, the development of a single starch grain in their central cavities, and an acquisition of the typical green color. The limiting factor to the greening of the plastids seems to be light rather than the size of the plastid. Fairly large plastids in the bases of the leaves where they are shaded lack pigment; yet those plastids in the tips of the leaves, while they are only slightly larger than those in the bases, have the color of mature plastids. That point in the development of plastids where they cease to become rod-shaped when fixed with bichromate fixatives comes later in Elodea than in Lunularia. In the leaves of the former it does not occur until after the plastids become capable of storing starch; and as a result a starch grain, often of considerable size, is frequently found in the center or end of a darkly staining rod. The plastid in this stage frequently resembles a spore-forming bacterium (Pl. LIII, fig. 11). If the included starch grain is very large the plastid will appear as a darkly staining crescent attached to one side of the grain (Pl. LII, figs. 8, 9).

Tracing the development of rod-shaped primordia into mature plastids is easier in the epidermis of the stem, for here the whole development is compressed within a few cells. While the primordia may be scattered throughout the cytoplasm, as a rule they seem to cluster about the nucleus (Pl. LI, fig. 5; Pl. LIII, fig. 12), an arrangement frequently reported for both primordia and mitochondria. Figure 8 shows plastids in several stages of development. The rod-shaped primordia are in the row of epidermal cells extending down from the center. These shade off at their upper end into small, starch-containing plastids, here crescent-shaped. The larger crescents are in the cortical cells of a node.

The starch has an index of refraction very close to that of the balsam in which the section is mounted and is consequently invisible. It can easily be seen, however, if the section is examined in polarized light (Pl. LII, fig. 7).

In the plerome the plastid primordia develop into the large chloroplasts which are specialized for the storage of starch and which occur in the cortex of the stem and in the layer surrounding the midrib of the leaf. In the living plastid the starch occurs in approximately the center of the plastid, though when fixed it appears to be eccentric, as in figure 9. The primordia in the periblem develop into the very small chloroplasts characteristic of the central cylinder.

ZEA MAYS

The present investigation of the growth and development of the plastids in Zea is limited to the changes which occur in seedlings of various ages.

The larger problem, as to whether the primordia are always self-perpetuating and are passed down from one generation to the next as permanent cell organs or whether they are formed *de novo*, can not be solved by such a partial investigation. It was possible to demonstrate the presence of plastid primordia in the embryo and in the seedling just as the plumule begins to emerge from the seed. The technique used here, however, was not adequate to allow the complete story of plastid development in the seed or very young seedling to be worked out, for here the problem is complicated by the presence of a quantity of reserve protein food. In the living cell this obscured the small, colorless plastid primordia and in fixed material it appeared in globules of various sizes ranging in a single cell from a diameter of 4 μ to the limit of visibility (Pl. LIII, fig. 13). These globules occur in all of the cells of the plumule and radicle, and react to stains as plastid primordia do. In certain cells it is possible to distinguish them from the primordia when the material is fixed with Erliki's solution which causes the primordia to become rod-shaped while the protein bodies remain globular, but it is not always possible to make this distinction because of the minute size of the two inclusions. The fact that these globules, shown in figure 13, are protein in nature and not plastid primordia can be proved by fixing the plumule with Carnoy's solution, which dissolves out of the cell all lipoids, mitochondria and plastid primordia yet leaves these globules behind. By the time the seedling is four or five days old with a plumule from one inch to an inch and a half long, few or none of these globules remain to complicate the study of the plastid primordia.

Plastid primordia which were self-perpetuating were found in all of the cells of the meristem of these seedlings. Division can actually be seen. It may well be, however, that in some other stage of the life cycle the primordia are formed *de novo*.

It has long been known that certain plastids do not develop completely in the absence of light. It is obvious that certain ones in a very young seedling which has not yet reached the surface of the ground cannot be fully developed no matter what their potentialities may be. To obviate this difficulty seeds were grown under conditions which insured a maximum amount of light for the embryo. The seeds were first soaked twelve hours. Then a hole was pierced in each at the tip of the embryo, and the integument over the embryo peeled off. The seeds were next placed in a moist chamber on moist filter paper, embryo side up, and the chamber put in an unshaded spot in a greenhouse. For purposes of comparison seedlings were grown in the usual way. Considerable difference was found in certain plastids between seedlings subjected to these two treatments.

In the primary meristem of the four-day-old seedling the primordia occur as small prolate spheroids. The size of these primordia varies somewhat in different plants, their average long diameter being .75 μ. Unlike the protein globules, the size of which varies greatly within the cell, these primordia in a given cell are all of approximately the same size. There is no grading off to the limits of visibility; if they originate *de novo*, therefore, they must suddenly appear full grown. While the primordia in the apical cells of a single plant are thus of much the same size, they are as a rule somewhat larger in the corresponding cells of the older seedlings, though at no time do they possess any trace of color. When fixed with Erliki's solution they become rod-shaped, their length is unaltered, but their diameter becomes .25 μ. Sometimes all of the primordia in an entire stem growing point are of one size, though more commonly their size has appreciably increased (1.25 μ × 50 μ) when seen three cells back from the apex. As a rule the primordia are scattered throughout the cytoplasm, though before cell division they collect in two groups on either side of the nucleus (Pl. LIV, fig. 15). When slides of the older leaves prepared to show mitotic figures are examined in polarized light, starch grains can be observed grouped about the two poles of the spindle in the same way that the primordia are grouped. Evidently at cell division the primordia and the plastids are divided into groups of approximately equal size, though no evidence was found which would indicate that the division is as precise as that of the chromosomes.

That these small colorless primordia in Zea differ markedly from the mature plastids in more respects than mere size is shown by their reaction to fixatives. Just as in Lunularia and Elodea they are dissolved by acetic acid fixatives and made rod-shaped by those containing bichromate. In one respect, however, they are essentially like mature plastids. If the seedling four or five days old has grown under optimum light conditions *each of the primordia in the apical meristem will contain a single minute starch grain about .5 μ in diameter*. Thus even in this simplest stage the primordia are hollow spheroids of essentially the same structure as mature plastids. The method of demonstrating the presence of these minute starch inclusions is simple. It is only necessary to use a fixative which will dissolve the anisotropic lipoids, to stain lightly so as not to obscure the starch, to mount in a medium which has approximately the same index of refraction as starch, and to examine in polarized light. The starch grains appear as minute brilliant disks against a black background, each disk being divided into quarters by the two black interference lines which cross at the hilum. When light from a powerful source is used, such as a carbon arc or the glowing filament of a 100-watt bulb, the effect is much like that of the ultra microscope.

These starch grains occur in all of the cells of the meristem, though they are on the whole more numerous in the outer layers. Their number varies from twenty to thirty per cell. At the base of the stem growing point, just above the point where the leaf rudiments emerge, the starch grains are about 1 μ in diameter. It may be significant that in this stage the grains in a single cell are all of approximately the same size. In certain plants it is possible to trace the entire plastid growth by means of the included starch grains.

Tracing the development of these primordia in the various tissues derived from the stem growing point is somewhat complicated by the fact that the younger leaves which enclose the stem apex grow in a spiral, and thus any longitudinal section of the plumule will be a diagonal section of many of the leaves. For this reason it is often impossible to trace a single row of cells from the base to the tip of a leaf. This, however, does not prevent one from getting a very good picture of the general trend of development.

All of the cells formed from the primary meristem contain plastid primordia. In the chlorenchyma they develop into the normal chloroplasts (Pl. LIV, fig. 19). Development proceeds more rapidly, however, in those chlorenchymatous cells adjoining the vascular strands. Frequently these chloroplasts, which are the first to become green, will have a diameter twice that of those in the rest of the chlorenchyma. Primordia also occur in the procambial strands and in the cells of the vascular tissue. Here their complete development is inhibited and they remain colorless and rod-shaped when fixed (Pl. LIV, fig. 18). When several of these primordia are joined end-to-end they are indistinguishable from mitochondria. What function they serve here is uncertain. As far as is known they may not function at all and be only relics of a previous evolutionary stage when the vascular tissue was not so highly specialized. It is perhaps significant that certain tissues which have lost their ability to support photosynthesis, such as, fungal hyphae, contain numerous mitochondria. The epidermal cells of the mature leaf contain neither plastids nor primordia in spite of the fact that the dermatogen is rich in primordia. On the other hand they contain many mitochondria (Pl. LIII, fig. 14), as has been noted by Randolph (20). These mitochondria can be traced back to the growing point and seem to be merely undeveloped or degenerate plastid primordia; in fact, the derivation of mitochondria from plastids has already been reported by Emberger (7) and Friedrichs (8). The development of mitochondria from plastid primordia can also be traced to the growing point of the prop-rootlets which are derived ultimately from the stem growing point (Pl. LIII, fig. 16). These latter mitochondria seem to be chains of primordia joined end-to-end, and are capable of developing back again into plastids. This change can be followed by noting the transitional stages in any line of cells back from the root tip. The first noticeable change in the mitochondria during this transformation is an increase in their diameter (Pl. LIV, fig. 17). Sometimes they develop small vacuoles (Pl. LVI, fig. 24) which, unlike the vacuoles in the primordia of the stem growing point or leaf, do not contain starch. The transformation ot mitochondria to plastids in the prop-rootlets does not take place with equal rapidity in all of the cells, being much slower in the narrow cells which surround the water ducts than in the cortical cells (Pl. LV, fig. 21). There is also a difference in the speed of their formation in the inner and outer cortex, as the cortex within the vascular ring will contain mitochondria, while the outer will contain plastids. It is possible to find mitochondria, transitional stages, and plastids within a single transverse section of the rootlets (Pl. LVI, fig. 24). Various transitional forms are shown in figures 6 and 22–25. A better place to trace the development of mitochondria into plastids is not in the rootlets but in the sub-epidermal layer next to the dorsal surface of the plumule sheath. A single longitudinal section five mm. in length will show all of the transitional stages.

This difference in the plastid development in stem and root was quite unexpected. In the stem growing point the primordia are essentially tiny plastids; and in the root tips, fixed with mitochondrial fixatives, they cannot be distinguished from mitochondria. This difference can be explained if we make two assumptions. First, the plastid primordia may be able to reproduce by division during several different stages of their development into plastids. Such division has, indeed, been reported frequently, numerous workers even reporting division in mature plastids (Kiyohara 13, Kassman 11, and others). Second, we may assume that it is the reproduction rather than the growth of the primordia which varies with the rapidity of cell division, since in this case we should expect smaller primordia when cell division is more rapid. Now cell division is much more rapid in the primary meristem of the rootlet than in that of the stem. The rootlet of the plant four or five days old is not only several times as long as the plumule, but, in the former, cell division is concentrated in the tip, while in the latter it occurs throughout the bases of the leaves as well as along the stem below the point where the leaves emerge. If we interpret the mitochondria in the rootlet, then, as very small primordia joined end-to-end and made rod-like by fixation, plastid development in root and stem is essentially the same.

In the hypocotyl and epicotyl plastid development is much like that in the root, except that in the cells of the vascular ring the primordia remain in Streptococcus-like chains which never develop into plastids and which, when fixed, simulate mitochondria. In the central cortex the mature plastids contain, as a rule, a single large, simple starch grain. In the exterior cortex the starch inclusions are in compound grains. There are, of course, exceptions to this generalization. In the mature plastid the compound starch grain is contained in the central cavity just as is the simple starch grain. The two types of grains, however, have different origins. The plastid which develops a simple starch grain has but a single vacuole. Starch forms in this vacuole in successive layers about a single hilum. Young plastids which later contain compound starch grains have several small vacuoles, in each of which a minute starch grain is formed (Pl. LV, fig. 20). As the plastid matures, the several vacuoles fuse and the included starch grains grow together to form a compound grain.

Light, of course, is necessary for the development of chlorophyll in the plastids though it is by no means the only factor involved, for certain plastids remain colorless for several days even after they have received more light than neighboring ones which have become green. Certain shaded plastids never developed pigment; though whether this lack of color was due to their composition, or to the fact that their position in the plant was one which precluded the access of enough light, could not be ascertained in the majority of cases. In some instances, however, it could be shown that plastids normally colorless become green when exposed to light; in others, such plastids remain colorless. For example, the plastids in the hypocotyl of seedlings grown from planted seeds were colorless; but in seedlings grown from seeds exposed to light in moist chambers, the plastids in certain of the cells of the hypocotyl became green before any of the leaves in the plumule. This greening took place not in the surface cells, as might have been expected, but in the cortical cells on both the inner and outer surface of the vascular ring. A cross section of the hypocotyl thus shows two concentric green rings. The plastids in the cortical cells nearer the epidermis, though they receive more light and are just as large as those next to the vascular ring, remain colorless for approximately a week after the latter have become green; in some instances they never develop color.

The first plastids to become green in the seedlings grown both from planted seeds and from seeds grown in moist chambers are in the subepidermal layer of the plumule sheath. In the moist-chamber seedlings the next plastids to become green are in the hypocotyl, as has been stated. The first leaf to become green in both types of seedlings is the outer one, located just within the plumule sheath. In the four-day-old seedling all of the leaves are distinctly green except the two immediately adjoining the stem growing point. The outer of these two leaves usually is distinctly yellow, but the growing point itself, and the leaf immediately surrounding it, never show any signs of pigment. The entire blade of the leaf does not become uniformly green. The pigmented portion occurs in longitudinal strips, which are composed of cells surrounding the vascular bundles, the bundle itself containing no pigment. The white strips are composed of the mesophyll farthest removed from the bundles. They are wider in the outer leaves of the young seedling and as a result the inner leaves regularly appear greener. The greening of the plastids does not seem to be closely correlated with their size, since some of the plastids in the outer leaves will reach a diameter of 4 μ without becoming pigmented, while some of the plastids in the younger leaves are distinctly green when they reach a diameter of 2 μ.

As the seedlings grow the amount of light received by the growing point is continually lessened, for the number of leaves wrapped about the growing point increases and the leaves themselves thicken. The result is that the greening of the chloroplasts in the younger leaves is delayed. When the plant is 18 inches tall there will be from 6 to 10 of the younger leaves showing no trace of chlorophyll, though their plastids will frequently have a diameter exceeding 4 μ.

SUMMARY

The plastids of Lunularia are developed from primordia in the apical cell, which are not produced *de novo* but reproduce by division, the division occurring regularly in one plane only. These primordia are small, colorless, prolate spheroids which are soluble in fixatives containing acetic acid and are rendered insoluble in the dehydrating alcohols by the usual mitochondrial fixatives. When fixed they are rod-shaped and measure about 1.25 μ by .25 μ. In the cells immediately surrounding the apical cell the primordia have increased slightly in size and have become yellow-green. In the subepidermal layer, approximately 15 cells from the apex, the primordia undergo certain fundamental changes: they become grass-green; they are no longer soluble in fixatives containing acetic acid; and when fixed with mitochondrial fixatives they no longer become rod-shaped but remain typical lens-shaped plastids. Mature plastids in the principal photosynthetic tissue often reach a size of 5 $\mu \times 7 \mu$. Those deeper in the thallus are rarely over 3 $\mu \times 4 \mu$. When plastids are fixed with a bichromate fixative the pores connecting their central cavities with the exterior become so enlarged that the plastid appears as a darkly staining meshwork surrounding a starch grain. The cytoplasmic contents of mucilage-secreting cells are densely stained with the mitochondrial stains, Janus green B, etc. Neither plastid primordia nor mature plastids are stained specifically with Janus green B, although when mature plastids are placed in a solution of low osmotic value and the contents of their central vacuoles has exuded, the surface film of these contents can be colored distinctly with this stain. The young epidermal cells contain, in addition to a few plastid primordia, numerous lipoid droplets. When fixed with Erliki's solution and stained with Heidenhain's haematoxylin, the lipoid appears as yellow globules and the primordia appear as dark blue rods.

The apical meristem of Elodea contains numerous plastid primordia somewhat smaller than those in Lunularia. When fixed they measure .5 $\mu \times .25 \mu$. These primordia are frequently strung end-to-end and are often grouped about the nucleus. They enlarge, acquire pigments and develop into plastids just as in Lunularia. The mature plastid when fixed with a bichromate fixative becomes crescent-shaped with a large starch grain on its concave surface. Frequently immature plastids will contain starch inclusions and when fixed will be shaped like tennis racquets. The development of primordia into plastids in the axillary scales is inhibited. The scales never turn green and their cytoplasm contains numerous mitochondria.

The plastid primordia in the apical meristem in a four-day-old seedling of Zea are of the same size as those in Elodea. Sometimes these primordia contain minute starch grains .5 μ in diameter in their central vacuoles, as can be seen when they are examined in polarized light. They are essentially small plastids. When fixed with a bichromate fixative the primordia become rod-shaped. Before cell division they become grouped into two masses lying at opposite poles of the nucleus. In the mesophyll and cortical tissue of the leaf and stem the primordia develop into plastids. In the vascular bundles, the epidermal cells, and the tips of the prop-rootlets their further development is inhibited. They remain colorless, are rod-shaped when fixed, and when joined end-to-end are indistinguishable from mitochondria. In the rootlets these mitochondria-like bodies change back to plastids. The first plastids to become green are those immediately surrounding the vascular bundles. Green plastids will occur within the second embryonic leaf from the growing point in the four-day-old seedling. Often plastids 2 μ in diameter are quite green, while others which receive an equal amount of light and are 4 μ in diameter are colorless. As the seedling ages, the growing point and the inner embryonic leaves become increasingly shaded through the thickening and the increasing number of the leaves, with the result that the greening of the young plastids is delayed.

This work was done while the writer held an appointment as National Research Fellow in Botany. He wishes to acknowledge his indebtedness to Professor Edward M. East for encouragement and helpful criticism.

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DESCRIPTION OF PLATES

These photographs were taken with a Leitz "Maccam" camera. None are retouched except figure 11. The magnification is 800 times except that of figure 11, which is 1600 times. Unless otherwise stated the fixative used is Erliki's. The stain used was iron-alum haematoxylin. No counter stain was used.

PLATE LI

FIG. 1. Longitudinal section through growing point of *Lunularia vulgaris,* showing the rod-shaped plastid primordia in the apical cell, oil droplets and primordia in the epidermal cells and the developing plastids in the sub-epidermal cells.

FIG. 2. Longitudinal section of axillary scale of *Elodea canadensis,* showing mitochondria.

FIG. 3. Mature plastids of Lunularia showing enlarged pores due to the fixation.

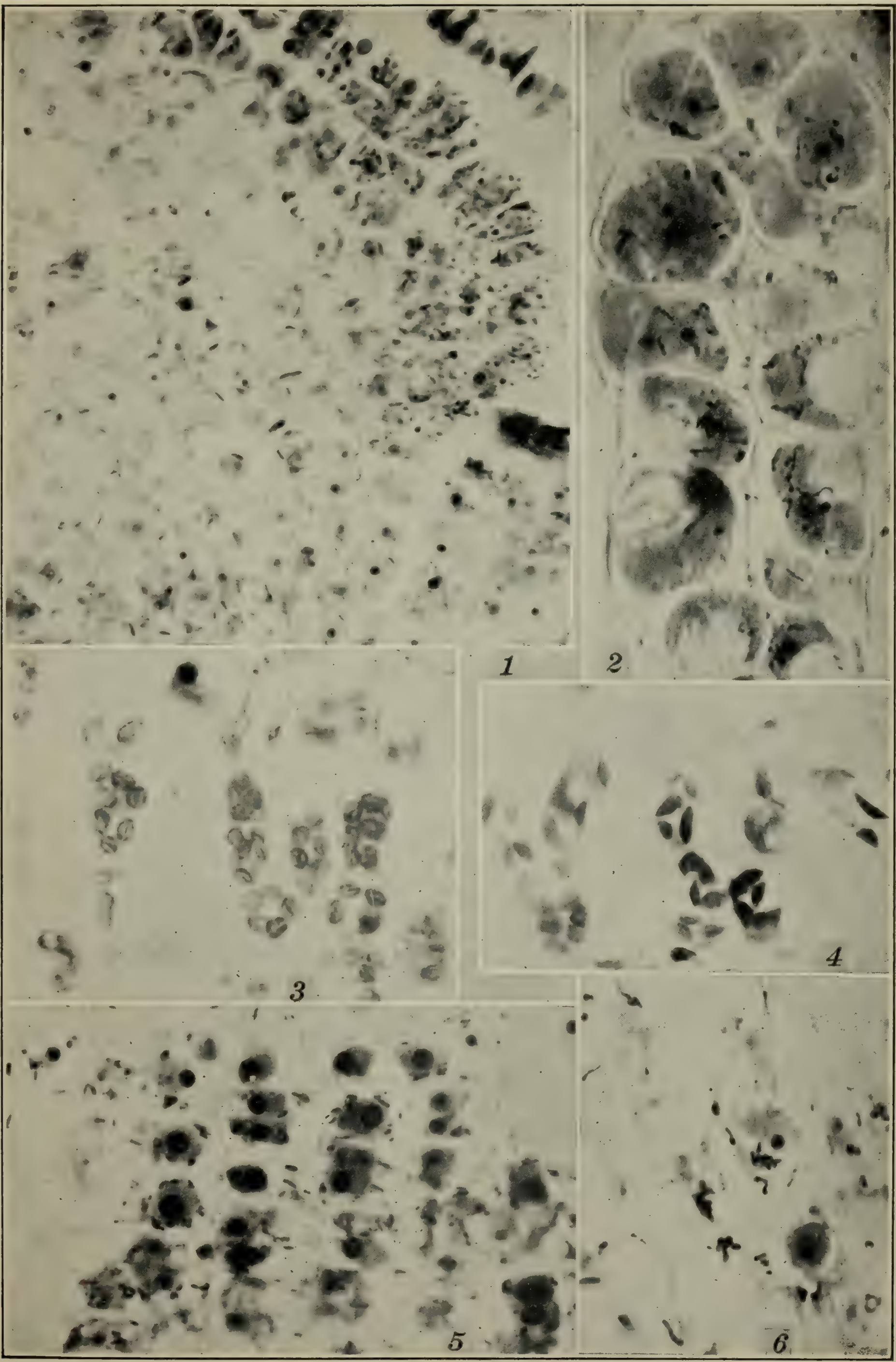
FIG. 4. Mature plastids of Lunularia fixed with Mottier's modification of Flemming's strong fixative.

FIG. 5. Slab section near apex of stem of Elodea, showing plastid primordia grouped about the nuclei.

FIG. 6. Cross-section of prop-rootlet of *Zea Mays,* showing plastid primordia.

PLATE LII

FIG. 7. Longitudinal section of stem of Elodea near the apex photographed in polarized light. The larger starch grains are at a node.



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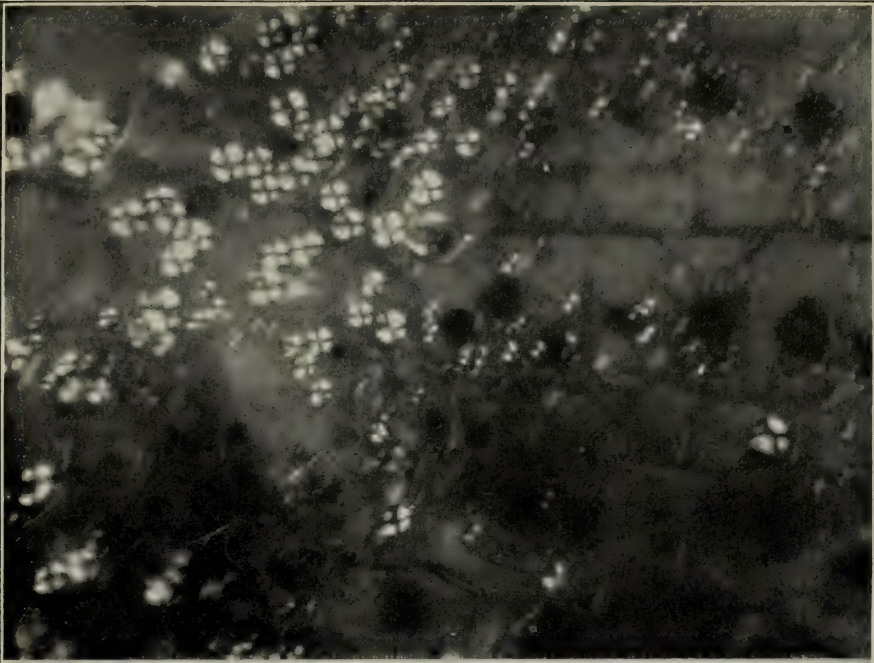

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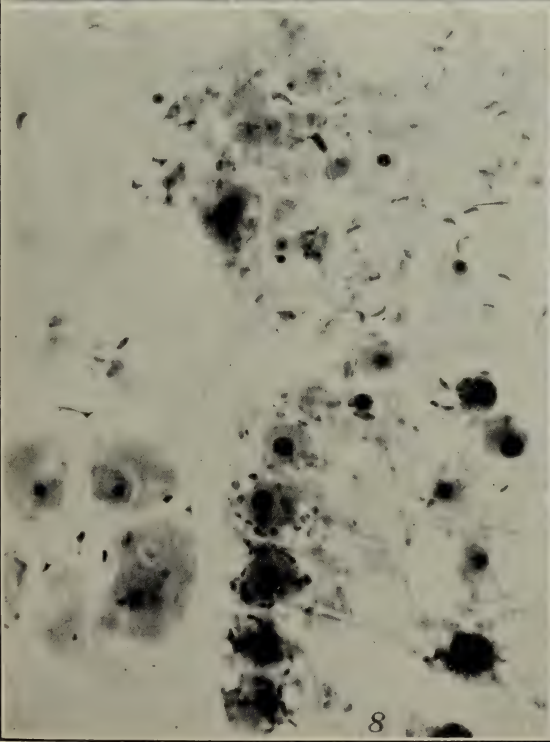

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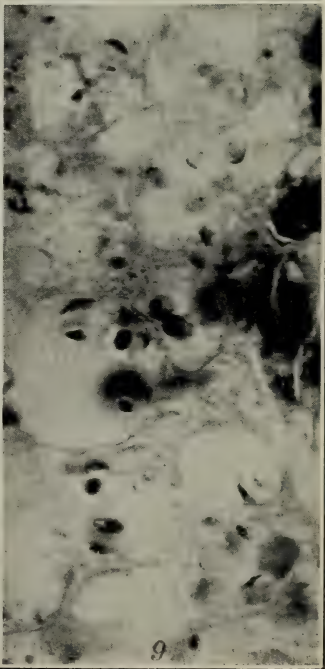

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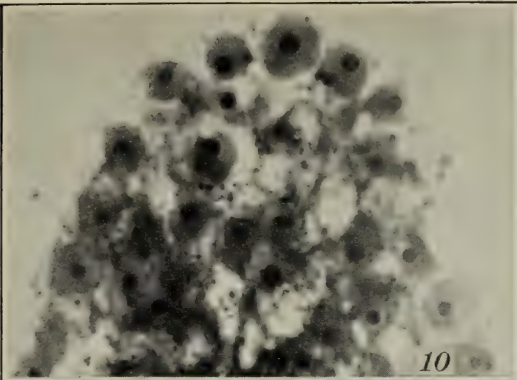
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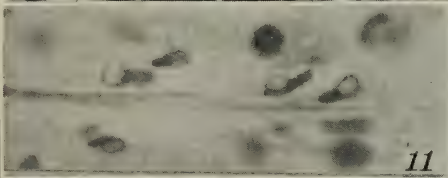
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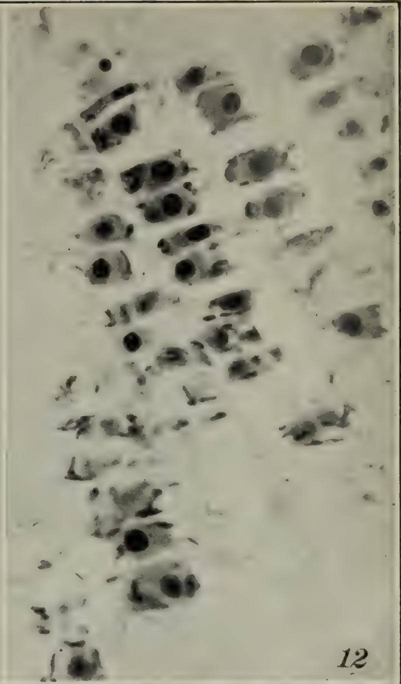
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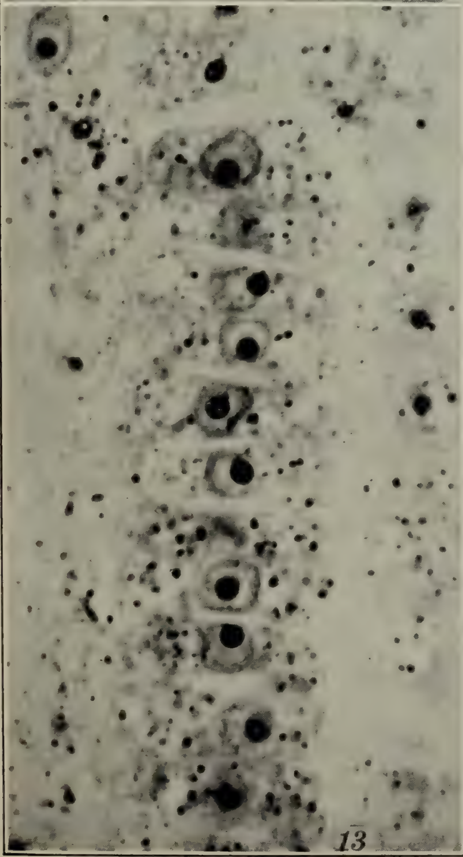
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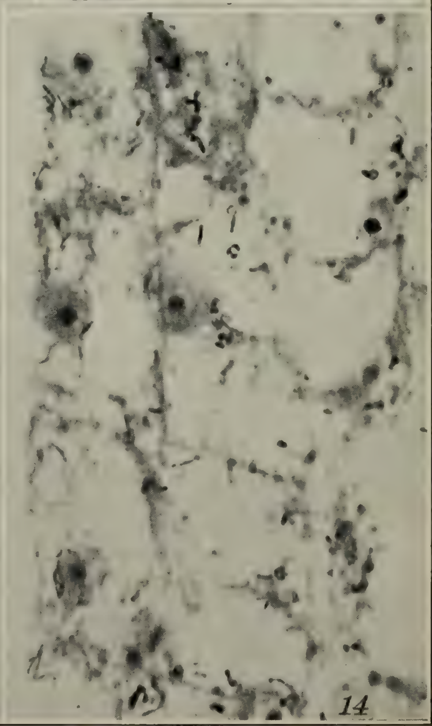
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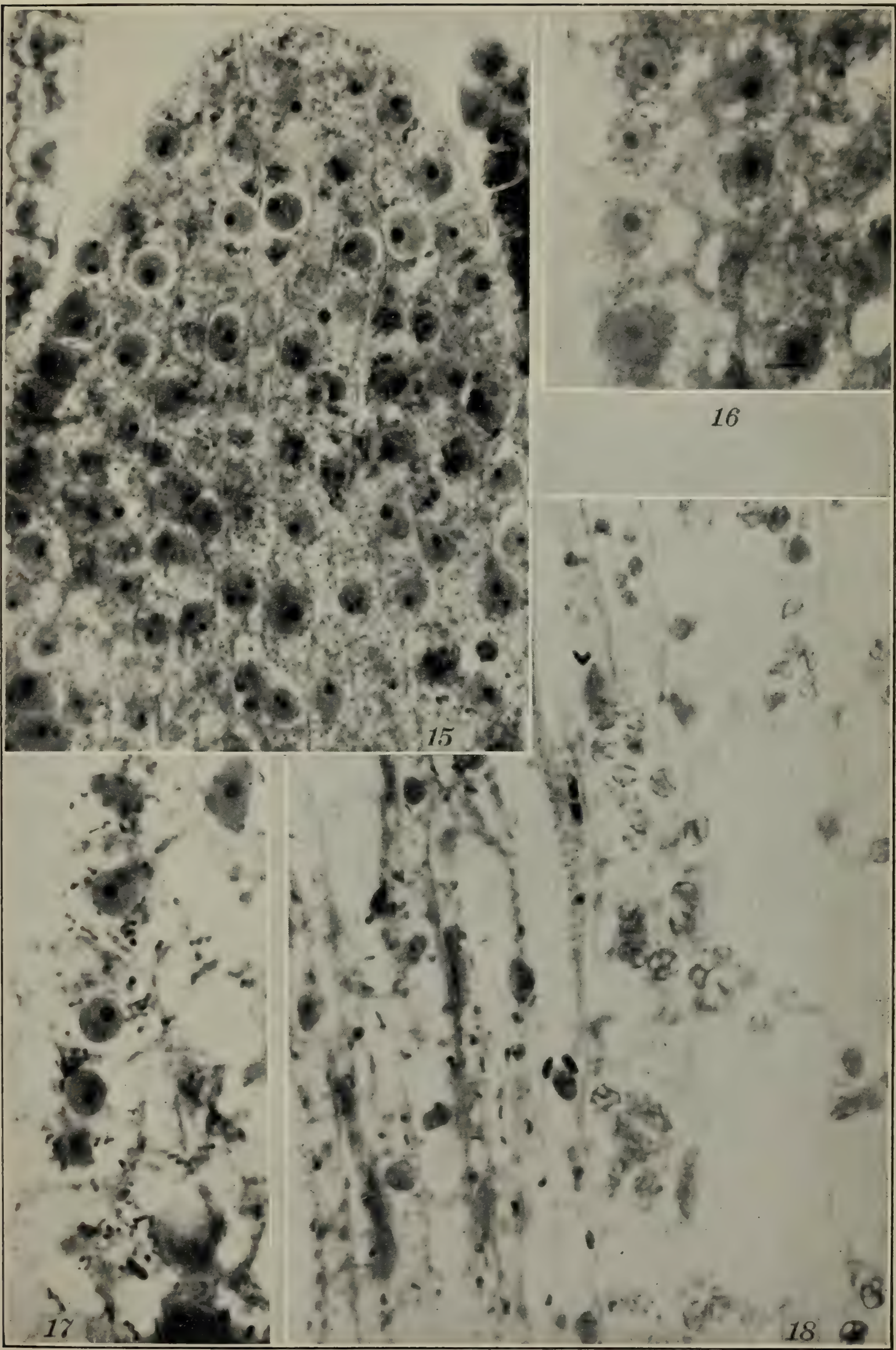

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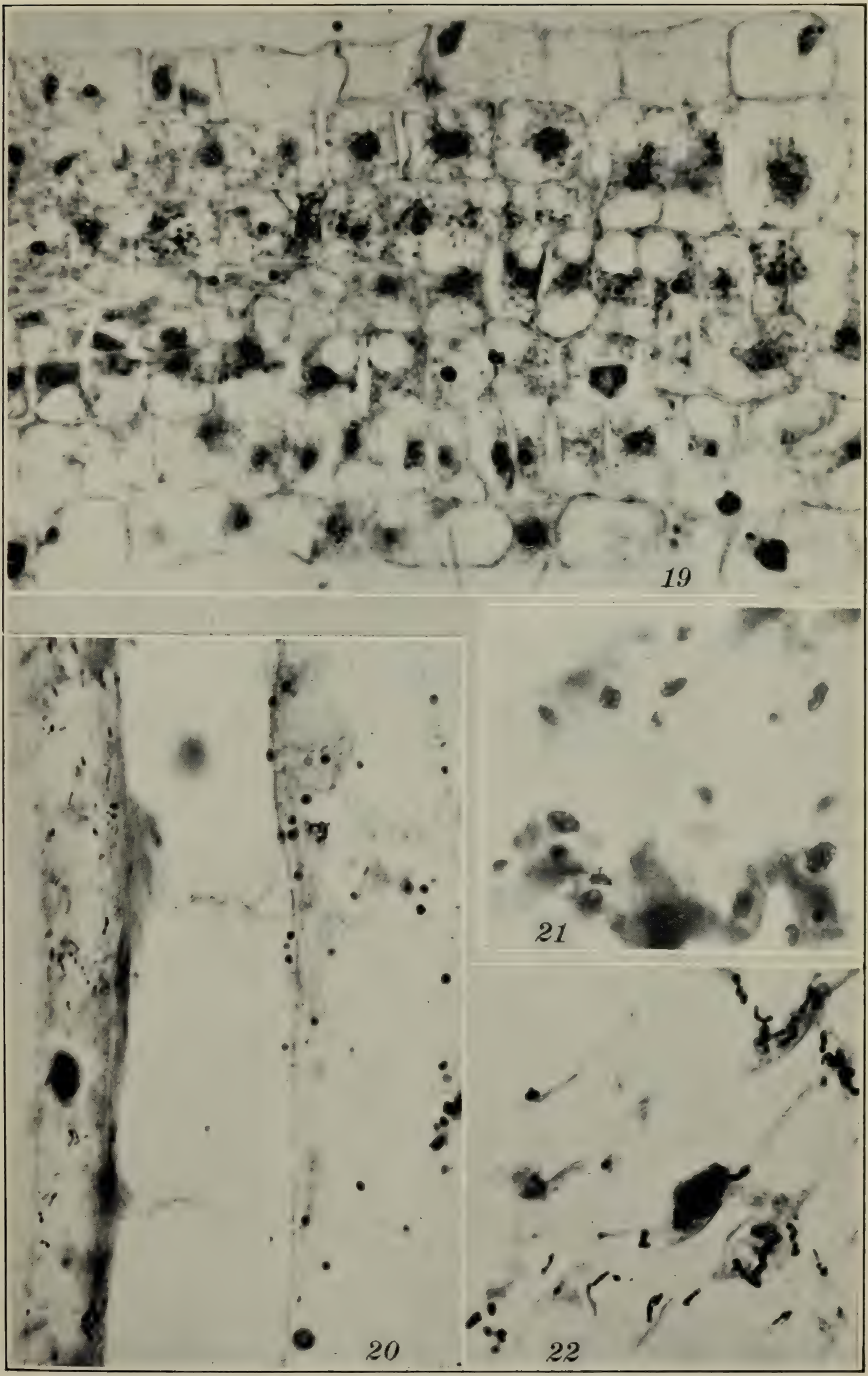

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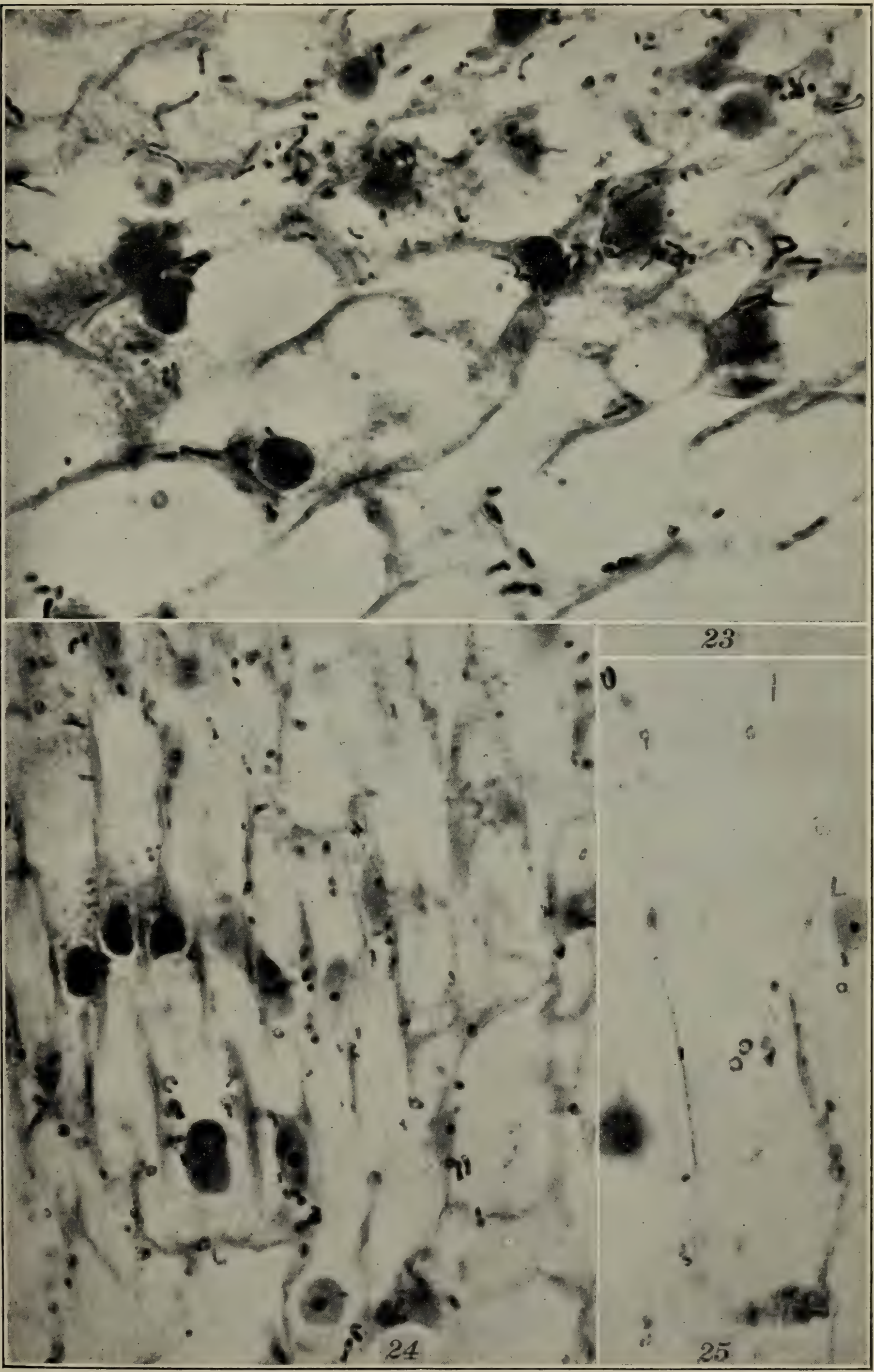

ZIRKLE: DEVELOPMENT OF PLASTIDS

FIG. 8. A portion of figure 7 photographed in normal white light. The darkly staining crescents are plastids each of which contains a starch grain on its concave surface. The row of epidermal cells in the center of the lower half of the figure contain rod-shaped primordia developing into starch-containing plastids.

FIG. 9. Large starch-containing plastids. The fixation has caused the plastid to become concentrated upon one side of the starch grain.

PLATE LIII.

FIG. 10. Stem growing point of Elodea, showing plastid primordia.

FIG. 11. Immature plastids containing starch grains in leaf of Elodea. × 1600. Retouched.

FIG. 12. Plastid primordia grouped about the nuclei in epidermal cells of the stem of Elodea.

FIG. 13. Cells near the base of an embryonic leaf of two-day-old Zea seedling. The cytoplasmic granules of various sizes are of protein reserve food-stuff. The plastid primordia have been dissolved by the fixative. Carnoy's fixative used.

FIG. 14. Cells from the longitudinal section of the plumule sheath of Zea. The epidermal cells on the left contain mitochondria, the sub-epidermal cells in the center contain transitional stages, and the third layer on the right, plastids.

PLATE LIV

FIG. 15. Stem growing point of five-day-old seedling of Zea. The granules in the cytoplasm are plastid primordia. In certain of the cells they are grouped on opposite sides of the nucleus. Such grouping precedes cell division.

FIG. 16. Cross section near the tip of a prop-rootlet of Zea. The plastid primordia can be seen grouped about the nuclei in the epidermal cells.

FIG. 17. Transitional stages of primordia in section of rootlet further back from the apex.

FIG. 18. Longitudinal section through a vascular bundle near tip of oldest leaf in four-day-old seedling of Zea. In the vascular tissue the plastid primordia have remained colorless rods. In the adjoining chlorenchymatous tissue they have developed into chloroplasts. The enlarged pores in the chloroplasts are due to the fixative.

PLATE LV

FIG. 19. Longitudinal section of the second inner embryonic leaf of five-day-old Zea seedling. The small chloroplasts appear crescent-shaped. When the section is examined in polarized light a single starch grain can be seen on the concave surface of each plastid.

FIG. 20. Immature plastids near base of plumule sheath. They are multiple-vacuoled. In each vacuole is a minute starch grain. The large multiple starch grains of Zea are formed by these grains growing together.

FIG. 21. Longitudinal section through a water duct near the tip of a large prop-root of Zea. The narrow vascular cell on the left contains mitochondria, the cortical cell on the right, plastids.

FIG. 22. Plastid primordia in slab section of prop-rootlet of Zea.

PLATE LVI

FIG. 23. Cross section of prop-rootlet of Zea. The cells at the top of the figure are in the vascular ring and contain mitochondria. The cortical cells at the bottom of the figure contain plastids. Various transitional stages can be seen.

FIG. 24. Various transitional forms between mitochondria and plastids in prop-rootlet of Zea.

FIG. 25. Other transitional forms.

INDUCED IMMUNITY IN PLANTS

BY

DONTCHO KOSTOFF

Bussey Institution of Harvard University

Reprinted from the Proceedings of the NATIONAL ACADEMY of SCIENCES,
Vol. 14, No. 3, pp. 236–237, March, 1928.

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Vol. 14, No. 3, pp. 236-237, March, 1928.

INDUCED IMMUNITY IN PLANTS

By Dontcho Kostoff

Bussey Institution of Harvard University

Communicated February 15, 1928

A series of experiments on the family Solanaceæ has apparently shown that plant tissues can acquire immunity against certain antigenic agents of other species by some type of antibody production.

Normal precipitins were found in leaf extracts and stem extracts and these were tested by Uhlenhuth's ring method (1909).

A great variability in amount of precipitation was observed when normal plant extracts were tested against each other. Powerful precipitation was observed both when the two extracts tested against each other were from plants of the same genus and when plants of different genera were used; but in certain combinations—particularly with different genera—instead of a precipitation ring, a lytic ring (a ring of liquid clearer than the two components) was observed.

The precipitation potency of certain species and genera is increased after grafting; moreover, in certain combinations whose extracts show no precipitin reaction before grafting, the capacity to produce precipitins is acquired during the growth of the graft unions. The highest acquirement of precipitins was found between 30 and 45 days after grafting. Acquirement of precipitins after grafting was observed in the following graft unions: *Nicotiana Rusbyi* grafted on *Nicotiana rustica*, *Solanum tuberosum* grafted on *Nicotiana rustica*, *Nicotiana Sanderæ* grafted on *Datura ferox*, *Nicotiana glauca* grafted on *Capsicum pyramidale*, *Lycium Barbarum* grafted on *Solanum Lycopersicum*, etc.

The precipitins acquired after grafting are usually specific. Thus extract of *Nicotiana Rusbyi* immunized against *Nicotiana rustica* by grafting always gave the heaviest precipitate when tested against its antigenic extract, namely, the normal extract of *Nicotiana rustica*. Then followed in order the extracts of *Nicotiana paniculata*, *Nicotiana glauca*, F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*) and *Nicotiana Tabacum*. It is perhaps important to note in this connection that the three species *Nicotiana rustica*, *Nicotiana paniculata* and *Nicotiana glauca* belong to the subgenus or section *Rustica*, while the species *Nicotiana Rusbyi* and *Nicotiana Tabacum* belong to the section *Tabacum* (Comes, 1899).

Acquired precipitins of *Solanum tuberosum* against (grafted on) *Nicotiana rustica* precipitate the normal extract of *Nicotiana rustica*, but not the normal extract of *Nicotiana Rusbyi*. Sometimes, however, the extracts of *Nicotiana Rusbyi* immunized by grafting against *Nicotiana rustica* showed increased precipitation potency over the precipitation shown against the normal extract of *Nicotiana alata*. The latter *Nicotiana* species belongs to the section *Petunioides* and the acquired precipitins of *Nicotiana Rusbyi* cannot be called "*specific*" but "*heterogenetic*."

In certain graft unions no acquirement of precipitins was found; viz., *Nicotiana Tabacum* grafted on *Nicotiana rustica*, *Nicotiana rustica* grafted on *Nicotiana quadrivalvis*, *Nicotiana Tabacum* grafted on *Solanum nigrum*, *Salpiglossis sinuata* grafted on *Datura ferox*, *Solanum Melongena* grafted on *Solanum nigrum*, *Nicotiana Tabacum* grafted on *Solanum Melongena*, *Nicotiana Tabacum* grafted on *Solanum tuberosum*, *Nicotiana glauca* grafted on *Nicotiana rustica*, etc.

A decrease of the normal precipitin potency was observed in the following graft unions: *Solanum tuberosum* grafted on *Datura Wrightii*, *Solanum Lycopersicum* grafted on *Datura Wrightii*, *Solanum Melongena* grafted on *Datura Wrightii*, etc.

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CYTOLOGICAL STUDIES IN THE GENUS NICOTIANA

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Bussey Institution, Harvard University, Boston, Massachusetts

Reprinted from GENETICS 13: 233-277, May, 1928

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CYTOLOGICAL STUDIES IN THE GENUS NICOTIANA

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Received November 23, 1927

TABLE OF CONTENTS

Introduction 233
Method and technique 234
The Chromosomes of the various Nicotiana species 235
Hybridization results 243
Description of the species hybrids 246
Cytological observations on certain Nicotiana species hybrids 254
DISCUSSION 258
  Inheritance in species hybrids 258
  Non-viability of the gamete in species hybrids 260
  Non-viability of the zygote in species hybrids 260
  Incompatibility between species in Nicotiana 262
CONCLUSION 263
SUMMARY 264
LITERATURE CITED 267

INTRODUCTION

The genus Nicotiana has been a favorite subject for study since the time of KÖLREUTER, his investigation of crosses between *N. paniculata* and *N. rustica* being the first genetical research of which we have a record. Unfortunately, the work of the hybridizers of the eighteenth and nineteenth centuries produced few generalizations of value to genetics, because of the numerous variables under consideration. The foundations of genetics have been laid by experiments where a limited number of genetic differences have been followed and where the fertility of the hybrids was virtually perfect. More recently, however, there has been a trend back to the study of species hybrids, using both the cytological and the pedigree culture methods of technique. The work of SAX on the genus Triticum is an excellent example. Using the general philosophy of genetics built up by intra-specific studies as a basis of his inductions, SAX has been able to throw considerable light , not only upon the genetic behavior of the species composing this genus, but also upon their origin. It was thought, therefore, that perhaps a similar series of investigations upon the genus Nicotiana might yield results of some value. There was the further hope that the genus might again be brought to the favorable attention of geneticists. It deserves a high place in their regard, for the plants are easy to grow, crosses are easy to make, large quantities of seed are produced, and the seed retains its viability for several years.

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[1] Fellow of The International Education Board.

Since the genus is fairly large (about 60 species having been described) it has been impossible for me to make even a relatively complete survey. I have been able to obtain 19 species. In each of these forms, the chromosome number has been determined. In addition, 15 species were selected for hybridization work, and an effort made to cross each of these species with all of the others. In the case of nine hybrids, the cytological behavior of the F_1 plants was investigated.

The species used correspond with the descriptions found in COMES' Monographie du genre Nicotiana (1899), with one or two exceptions. These exceptions are species described since the appearance of this work. Their descriptions can be found in the original publications cited in the bibliography.

METHOD AND TECHNIQUE

The haploid chromosome number in each species was determined both from aceto-carmine preparation made according to Bellings' method and also from permanent slides. A very small part of the bud was cut away at the tip so that a part of each anther could be examined by the aceto-carmine method. The rest was immediately fixed with modified Bouin's solution as developed by ALLEN. Sections were cut 7 to 10 microns thick, and were stained with Haidenhain's iron-hæmatoxylin.

The diploid chromosome numbers of the pure species, and in a few cases of the dwarf species hybrids, were determined by a study of the root tips at a time when the plant, grown in sterile soil, had produced its 6th or 7th leaf. The fixative in this case was strong Flemming's solution, the stain being again Haidenhain's iron-hæmatoxylin.

In the hybridization work, 3 or 4 plants of both species were always used. The parent plants were kept together, but isolated as far as possible from the plants of the other species. The hybridization was made in the greenhouse, and in order to prevent contamination, all buds which could not be used for hybridization were cut off at a very early stage. The flowers were not bagged. Flying insects were excluded from the house by cheesecloth covers; and experiments have shown that with plants having heavy pollen, as is the case with all Nicotiana species, this is a sufficient precaution. Crosses and reciprocals were made in each case using five buds of every combination. The seeds were always grown in sterilized soil.

THE CHROMOSOMES OF THE VARIOUS NICOTIANA SPECIES

Tabacum section

1. *N. Tabacum* L. Within this species I have made cytological studies of the following varieties: Cuba, *macrophylla*, and a white flowering tobacco. According to COMES, Cuba belongs to the var. *havanensis*. It has lanceolate leaves and pink flowers. *Macrophylla* is a very strong plant with large ovate leaves gradually tapering to a broad clasping base, with rounded but not prominent basal lobes. The flowers are deep red with a stout tube and an abruptly swollen, broad infundibulum; the limb is almost pentagonal, and at the base of every lobe is a whitish, triangular spot. The white tobacco is a small variety bearing a large number of small lanceolate and broadly decurrent leaves tapering towards the base on a relatively short stem. The corolla is relatively small, short, and cream colored.

In each of these three varieties, I found 24 chromosomes in the pollen mother cells during the different stages of the reduction division. The number was very easy to count at the second metaphase or at the first anaphase because the chromosomes at these stages, though small, are clearly separated. Sometimes the first metaphase (figure 19) is exceptionally clear and favorable for the study of chromosome size. At this stage the chromosomes are twice as large as in the second metaphase and lie close together. Figure 19 shows clearly the differences in the chromosome size. The small chromosomes are arranged in the center, the larger chromosomes at the periphery.

There is great difference in the chromosome shape at meiosis and at mitosis. In the cells of the root tips the chromosomes are long and slender, usually curled, and difficult to count. In figure 1 is represented one of the root tip cells of *N. Tabacum* var. *macrophylla* in which it is relatively easy to distinguish 48 chromosomes.

Rustica section

2. *N. rustica* L. In this species I have studied the varieties *humilis*, *brasilia*, and one which is apparently *texana*. The 24 haploid chromosomes of the pollen mother cells, in contrast to those of *Tabacum*, are uniform in size and shape. Their shape is markedly different from that of the *Ta-*

bacum chromosomes, being bean-shaped instead of spherical (figure 18). During the second metaphase they are to be seen clearly because of their separation, but are so small that their shape is vague, and they appear to be spherical.

The somatic chromosomes, though somewhat larger than those of *Tabacum*, are entwined and difficult to distinguish. It was impossible to make counts on preparations of root tips from the small *humilis* variety because the cells are very small and the chromosmes much entwined. Only in *brasilia* was I able to find clear plates (figure 2) where the chromosomes are manifestly 48.

3. *N. paniculata* L. The material used had all of the characteristics of true *N. paniculata*—a long, slender corolla tube with short, symmetrical, greenish corolla lobes. The upper leaves were lanceolate, the basal leaves were ovate, slightly cordate, and with long petioles.

The 12 chromosomes counted during different stages of the reduction division in the pollen mother cells (figure 25) seem to be identical in shape with those of *N. rustica*. In the cells of the root tips the chromosmes are very slender, long, and twisted; but 24 are easily distinguished (figure 8).

4. *N. glutinosa* L. The material used in this study was a small branched plant with small, cordate, long petioled, extremely glandular leaves. The flowers were short and cylindrical below, but suddenly swollen above into an irregular, obliquely one-sided funnel. The limb was bilabiate, the stigma and anthers being connivent just under the middle lobe of the upper lip. The color was light yellow tinged with red.

Twelve chromosomes were counted in the different stages of the pollen mother cells. The chromosomes are uniform in size and shape, the spherical shape giving them a greater resemblance to those of *N. Tabacum* than to the bean-shaped chromosomes of *paniculata* or *rustica*. They are only one-half as large as those of *paniculata*. During the first metaphase they lie very close together (figure 26), but are very clear and distinct in the first anaphase. The differences between the chromosomes of *glutinosa* and *paniculata* are visible also in the somatic cells, those of the former (figure 6) being shorter and slenderer than the latter.

5. *N. glauca* Grah. This species is a tree tobacco with long, petioled, glaucous leaves. The flowers are pale yellow with tubes similar to those of *paniculata*. The stem and leaves are bluish green. The chromosome number was found to be 12 in the pollen mother cells (figure 23) and 24 in the root tips (figure 9). The somatic chromosomes are extremely large, long, and entwined, but because of the large cells and nuclei they are well scattered and therefore are easily distinguished.

6. *N. tomentosa* R. et. P. The two year old plants are not yet in flower, but their general character shows them to be true *N. tomentosa* (*Lehmannia tomentosa* Spr.). The chromosome number was counted in the cells of the root tips and found to be 24 (figure 10).

Petunioides section

7. *N. Bigelovii* Wats. According to SETCHELL (1912) different varieties of *N. Bigelovii* exist. The plants used were small, their first leaves being slightly decurrent, nearly petioled, in fact, the later leaves becoming characteristically truncate, auriculate, and partly clasping at the base. The first leaves were more or less elongate deltoid, the later leaves more lanceolate. The corolla tube was narrow, and the corolla limb deeply divided into long pointed lobes. The corolla was pure white. Chromosome counts were made most easily during the first and second metaphases. During the first metaphase (figure 16) the chromosomes appear as bean-shaped objects about equal in size but not quite so large as those of *N. rustica* and *N. Tabacum*. In the root tips 48 chromosomes were counted (figure 3).

8. *N. viscosa* Lehm. (?). This species was raised from seeds obtained from a Gray Herbarium sheet labeled *N. viscosa*. At the same time I obtained seeds from another Gray Herbarium sheet labeled *N. attenuata*. Both groups of plants had the same morphological characters. Both lots may be *N. attenuata*, but I have labeled them *N. viscosa* (?) because I raised other specimens received from Dr. T. H. GOODSPEED as *N. attenuata* which differed somewhat from the first two lots. The latter group of plants was rather similar to *N. acuminata*, but very much smaller. These specimens, in fact, seemed to be much reduced acuminatas, and therefore correspond more closely to the taxonomic description of *N. atenuata*. I am not certain of the identification, however, for these species are quite similar to each other, and perhaps should be grouped together.

The pollen mother cells are nearly as large as those in *macrophylla*. The chromosomes also are quite large, lying close together during the first metaphase (figure 20) and more distant during the second. During the first and second metaphases as during the first anaphase I counted 24 chromosomes.

9. *N. nudicaulis* Wats. *N. nudicaulis* is a very small plant with dark green ovate leaves narrowly decurrent. The corolla is shorter and broader than in *N. trigonophylla*. The tube is somewhat brownish, the limb cream. The corolla lobes overlap.

The chromosomes of the pollen mother cells are not easy to count at any stage of the reduction division, being small and usually rather dumbbell shaped. In the very early first metaphase the chromosomes are somewhat more spherical, however, and 24 could be distinguished (figure 21). In the root tips, it is relatively easy to count 48 chromosomes, the chromosomes being slender and well separated (figure 4).

10. *N. suaveolens* Lehm. The plants used were branched, with the lower leaves ovate, sessile, clasping at the base and pointed at the tip. The upper leaves were sharply lanceolate and clasping. The corollas were ivory white, salver shaped and with long slender, non-infundibular tubes. The corolla limb is large with broad, shallow rounded lobes.

During the first metaphase of the pollen mother cells, the 16 chromosomes always lie close together (figure 22). They are most easily counted in the first anaphase or in the second metaphase. Cells were found with both plates of a first anaphase lying in the same section in such a manner that by focussing 16 chromosomes can be counted in each. In the first anaphase, the chromosomes are seen as curved rods or as spheres depending on their position. In both the first and the second metaphases they are more or less spherical. During the second metaphase they are more separated and are easy to count. The chromosomes in this species are relatively very small though the cells in comparison with those of the other species are very large. In the cells of the root tips 32 chromosomes could be counted. The chromosomes here are rather slender, comparatively short, and often U-shaped (figure 5).

11. *N. sylvestris* Speg. *et* Comes. In general habit as in leaf shape this species of the Petunioides section is very similar to certain *Tabacum* varieties. Its corolla is white, and very long and slender, the tube being inflated in the middle. The corolla limb consists of 5 broadly triangular lobes. The flowers ordinarily droop.

In the pollen mother cells during the heterotypic metaphase 12 spherical chromosomes, closely arranged at the plate, were counted (figure 24). The first anaphase and the second metaphase are the best for making counts, the 12 chromosomes then lying fairly distant from each other. In the cells of the root tip preparations the chromosomes are long, U-shaped, quite large, and spread out, so that it is easy to distinguish 24 chromosomes (figure 7).

12. *N. acuminata* Grah. This is a branched plant with petioled leaves, cordate below and lanceolate above. The coarse, spine-like glandular hairs on the surface and on the margin are characteristic. The corolla is white with a cylindrical tube and shallow, rounded lobes on the limb.

Twelve chromosomes were counted during the different stages of the reduction division of the pollen mother cells (figure 27). It is easier to count them at the second metaphase or the first anaphase. The chromosomes after the second division are long and U-shaped, somewhat similar to those of the somatic cells, though during the other stages they are almost spherical. In some other plants this is a common phenomenon; but in the rest of the Nicotiana species it apparently does not occur, the chromosomes after the second division ordinarily resembling those of the earlier stages.

13. *N. trigonophylla* Dun. This is a very small plant with broadly lanceolate leaves, narrow at the base and then expanded into broad, partly clasping auricles. The corolla is very small with a short, cylindrical tube. The corolla limb consists of five broad, obtuse, and shallow lobes. The flowers are a deep cream tinged with green.

In all stages of the reduction division in the pollen mother cells 12 chromosomes were found (figure 28). The chromosomes were all of about the same size, spherical, and distinctly spread, especially during the second metaphase. In the somatic cells of the root tip they are easily distinguished as 24 relatively straight and slender chromosomes (figure 11).

Seeds from a specimen at the Gray Herbarium were obtained under the name of *N. Palmeri* A. Gray. Plants raised from them exhibited no particular differences from *N. trigonophylla*, except that the corolla was lighter and the leaves more lanceolate than those of my *trigonophylla* plants. Twelve chromosomes were counted in the different stages of the pollen mother cells (figure 29). Permanent slides of the young root tips show 24 nicely spaced chromosomes (figure 12).

14. *N. longiflora* Cav. This is quite a tall plant with broad, lanceolate or oblanceolate leaves, coarsely bullate and rugose above, the epidermis smooth except for coarse, spine-like, glandular hairs on the surface and margins. It early develops a characteristically compact rosette of large, coarse leaves which lie flat on the ground, the rosette persisting for a considerable time before the flowering stalk develops. The corolla tube is extremely long and slender. The broad, spreading limb is deeply divided into five moderately broad, blunt-pointed lobes. During the first metaphase (figure 30) and the first anaphase I counted 10 chromosomes.

15. From *N. plumbaginifolia* Viv., a species closely related to *N. longiflora*, I have obtained good preparations of the root tips in which 20 well separated chromosomes of different shapes and sizes could be clearly distinguished (figure 13).

16. *N. alata* Lk. et Otto. The plants which I have investigated came from commercial seed, and showed great variability. Concerning their origin I know nothing. They may have been segregates from

crosses between *N. alata* and *N. Forgetiana*, since it is supposed that the different colored varieties of *N. Sanderae* have originated by crossing *N. alata* with *N. Forgetiana* (HEMSLEY 1905). Some of these plants, however, seem to be the pure *alata* type. I have investigated the pollen mother cells of about 25 plants differing in habit, shape and corolla color, making both acetocarmine preparations and permanent slides. In some plants I found 8 chromosomes in all of the stages. In other plants I obtained irregular results. It was doubtful whether the number was 8 or 9, because one of the chromosomal masses could be considered as two small chromosomes lying close together, or as end portions of a single chromosome. Since I found the somatic chromosomes to be very different in shape and size, I am inclined to accept the second hypothesis. In the cells of the root tips I could distinguish 16 chromosomes; one pair very long and S-shaped, one pair long and straight, 4 pairs U-shaped and of different sizes, one pair very short and rod-shaped, and a small pair almost spherical (figure 14). Since the difference in size is so extreme in the somatic cells, the relative size proportions ought not to disappear in the pollen mother cells even though the contraction of the chromosomes there is very great. Some spindles of the first metaphase show clearly that the chromosomes are mostly long and dumb-bell-shaped, though in polar view they appear almost spherical. I am inclined to believe, therefore, that the position of some of the 4 U-shaped chromosomes may be such as to present only the tips which could appear as separate chromosomes lying close together.

It may be possible, also, that this occurrence is due to an earlier split of some of the chromosomes as described by WINGE (1917) for *Chelidonium majus* and *C. majus* var. *laciniatum*. WINGE explained BONICKE's determination of the chromosome number in *Chelidonium majus* as eight instead of six by the observation that the chromosomes in the pollen mother cells of this species have a marked power of precocious division. He found that "in the early anaphase, the six chromosomes moving towards the pole are not only divided into two, but into four, thus resembling a *Sarcina*, and, indeed, it would seem as if still finer subdivisions were present, which renders it difficult to ascertain the true number of chromosomes." The splitting phenomenon he found to be still more frequent in var. *laciniatun* than in the typical *Chelidonium majus*, and concluded: "The fact is of considerable interest from a theoretical point of view, as the distinct division in the heterotypic anaphase shows that the chromosome must not be regarded as an indivisible unit, but as a complexity, in which an inner differentiation or duality is already present. And in all probability, it is also actually existent even when no outward and visible sign of division is discernible."

From the many plants in which I have studied chromosome number in the pollen mother cells I shall present some figures drawn from temporary slides made by the acetocarmine method. By this method I was able to distinguish the rod shape of the chromosomes not only in side view on the spindles, but also in polar view, for the acetocarmine fluid included between the slide and the cover glass allowed the chromosomes to move slightly and to take different positions during the observation. In cases in which questionable numbers were found, the final conclusion was drawn only after exact observations of the questionable chromosomes in different positions. In figure 34, one plate of the second metaphase was in polar view, and appeared to have 9 chromosomes; the other was not exactly in polar view, and only 7 chromosomes could be counted with certainty. After standing, however, the position of the material was changed, the plate with the 7 chromosomes appearing as a regular spindle in which the chromosomes were more or less indistinguishable, and the other plate showing clearly 8 chromosomes.

In all of the other cells counted the number of the chromosomes was always 8 (as in figure 35). Figure 36 shows the shape of the chromosomes of the first metaphase in side view. In figure 32 all chromosomes clearly show splitting, and are similar in shape to those in figure 21*b*, *d*, and *h* presented by WINGE for *Chelidonium majus*. Figure 33 is very similar to figure 21*i* of WINGE. The chromosomes can be counted as 10, but since in two places chromosomes lie so close together, I am inclined to believe that they are two long chromosomes instead of four.

GOODSPEED in a preliminary note (1923) has reported for different Nicotiana species the haploid numbers 9, 12, and 24, but for some of them he makes the following reservation.

> Three of these counts are open to some question. In the case of *N. alata*, homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available, and there is some doubt as to whether the number is not larger than 12, possibly 18.

Later, (1924) he describes a very interesting case of *N. alata* in which, after the second division, two of the anaphase plates have 8, and the other plates 10 chromosomes. Failure of conjugation in one chromosome pair, he believes, will account for this condition, because in other cases he found

9 chromosomes in three nuclei of the tetrads. For *N. suaveolens*, he decides that the number is 18. At the IVth International Congress of Botany at Ithaca, I showed Dr. GOODSPEED on one of my slides a single section in which both the plates of the homeotypic anaphase contained only 16 chromosomes. In a recent publication (GOODSPEED and CLAUSEN 1927), this number is admitted to be the correct one.[1]

17. *N. Langsdorffii* Weinm. This is a small plant with sessile, elliptical-lanceolate leaves, narrowed and decurrent at the base. The corolla is funnel-shaped below with a gibbous ring above and a concave, spreading limb slightly notched into five broad, shallow lobes, greenish yellow and pendent.

At the various stages, 8 chromosomes were observed (figure 31), though it is not always easy to count them for reasons similar to those discussed in the case of *N. alata*. In the root tips (figure 15), I found 16 chromosomes similar in shape to the chromosomes found in the root tips of *alata* (figure 14).

Polydiclia section

18. *N. quadrivalvis* Pursh. This is a small plant somewhat similar to *Bigelovii*, except that the leaves are not elongate deltoid but broadly deltoid. The corolla is shaped somewhat similarly to that of *Bigelovii*, but is much larger. The capsule is spherical and twice as large as that of *Bigelovii*. During the various stages of the reduction division in the pollen mother cells, 24 chromosomes were easily counted (figure 17).

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[1] Since this paper was finished, Miss RUTTLE has published an interesting study of the chromosomes in *N. alata* var. *grandiflora* (Univ. of California Pub. Bot. **11**: 159–176, 1927). She has gone about her investigation in a very workmanlike manner, making extensive counts and even endeavoring to identify the individual chromosomes in various plates. She concludes that there are 18 chromosomes in the somatic cells, the usual distribution being 9–9, though 8–10 distributions occur in about nine percent of the cases. She believes that in my previous work (Jahrb. d. Univ. Sofia, Agric. Fac. **3**: 37–86, 1925) where a diploid number 16 was reported for the species, I incorrectly figured two chromosomes as one—a very long one. It is possible that Miss RUTTLE's view is correct. But I must call attention to two points which should be weighed carefully before a decision is made. First, Miss RUTTLE figures 3 pairs of U-shaped chromosomes in her Figure 1a plus 2 pairs the ends of which lie very close together (upper center). This latter configuration I believe to be a single pair of chromosomes. Second, and this is my critical point, in certain hybrids with species having 10 chromosomes as the haploid number, the reduction division of the pollen mother cells shows the number of univalents properly to be expected if the chromosome number for *N. alata* is $x=8$. For example, in heterotypic spindles of *N. longiflora*×*Sanderae*, *N. longiflora*×*alata*, *N. plumbaginifolia*×*alata*, *N. plumbaginifolia*×*Langsdorffii*, *up to two lagging univalent chromosomes* occur and are often to be seen approaching the poles, whereas the bivalents are still at the equator (see figure 37); and *regularly more than two lagging chromosomes* appear during the homeotypic division.

19. *N. multivalvis* Lindl. This is a plant very similar to *quadrivalvis*, only larger, with very large corolla and capsules. Counts were made only from temporary slides with actetocarmine staining and 24 chromosomes were counted in the pollen mother cells.

HYBRIDIZATION RESULTS

In the hybridization experiments the following species of Nicotiana were used:

1. *N. alata*
2. *N. Sanderae*
3. *N. longiflora*
4. *N. plumbaginifolia*
5. *N. acuminata*
6. *N. trigonophylla*
7. *N. sylvestris*
8. *N. suaveolens*
9. *N. Bigelovii*
10. *N. nudicaulis*
11. *N. Langsdorffii*
12. *N. paniculata*
13. *N. glutinosa*
14. *N. rustica*
15. *N. Tabacum macrophylla*

The technique of hybridization has already been described. Crosses were made between nearly all of these species in both directions, pollinating five (or more) flowers in each case. The few exceptions were the omission of *N. Sanderae* as female, of nine combinations with *N. nudicaulis*, and of one combination with *N. rustica*. The results obtained are recorded in table 1.

Two weeks after the capsules were harvested the seeds were sown. For every cross several sowings were made according to the quantity of seed harvested. If all of the flowers which were pollinated produced capsules, 100 seeds were first taken for the germination test, the rest being sown in sterilized soil for the production of F_1 plants. For crosses from which one or two capsules only were harvested, or in which only a few seeds developed, the material was used in the germination experiment. If germination occurred, the seedlings were transferred to pots of sterilized soil. If no germination occurred after three weeks, successive sowings were made until all the seed was used. The germination experiment was made in the usual way on blotting paper, the papers being kept in an incubator at a temperature of 27° to 30° C. Sometimes it happened that not a single seed germinated in the soil while a few germinated in the germination test; but it also happened that a few germinated in the soil though none appeared in the germination test.

A prospectus of all the crosses is given in table 1. In the horizontal rows are given the species used as female, and in the vertical row those used as male. The chromosome number for each of the species follows the name. The crosses in which the flowers have dropped after pollination are de-noted by *o*, those in which the harvested capsules contained no seeds or non-germinative seeds by *d*, those which produced defective seedlings by *s*, those in which mature hybrids were produced by *h*.

TABLE 1.

Results of the hybridization experiments.

SPECIES USED AS MALES		SPECIES USED AS FEMALES														
		N. alata	*N. Sanderae*	*N. Langsdorffii*	*N. longiflora*	*N. plumbaginifolia*	*N. acuminata*	*N. sylvestris*	*N. trigonophylla*	*N. paniculata*	*N. glutinosa*	*N. suaveolens*	*N. Bigelovii*	*N. nudicaulis*	*N. rustica*	*N. Tabacum*
		X=8	8	8	10	10	12	12	12	12	12	16	24	24	24	24
N. alata	X=8	..	..	h	h	h	d	d	d	s	o	s	d	d	..	h
N. Sanderae	8	h	..	h	o	o	o	d	d	h	d	o	d	..	h	h
N. Langsdorffii	8	h	..	..	o	h	o	o	s	h	s	o	s	d	h	;
N. longiflora	10	o	..	o	..	h	d	o	d	s	d	h	s	..	d	s
N. plumbaginifolia	10	o	..	o	h	..	d	o	d	s	d	h	s	..	d	s
N. acuminata	12	o	..	o	o	o	..	o	o	o	d	d	d	..	d	o
N. sylvestris	12	o	..	o	o	o	o	..	o	d	h	o	s	o	o	h
N trigonophylla	12	o	.	o	o	o	o	o	..	d	o	o	o	h	d	o
N. paniculata	12	o	..	o	o	s	o	o	d	..	d	o	s	o	h	o
N glutinosa	12	o	..	o	o	o	o	o	d	h	..	h	h	..	d	h
N. suaveolens	16	o	..	o	o	o	o	o	d	s	d	..	h	d	s	d
N. Bigelovii	24	o	..	o	o	o	o	o	d	d	h	h	..	..	d	d
N. nudicaulis	24	o	..	o	..	..	o	..	d	d	h	o	h	..	d	d
N. rustica	24	o	..	o	o	o	o	o	d	d	o	o	o	d	..	o
N. Tabacum	24	o	..	o	o	o	d	h	d	d	d	s	h	s	h	..

Combinations marked .. were not made
Crosses marked o produced no capsules
Crosses marked d produced capsules without viable seeds
Crosses marked s produced defective seedlings
Crosses marked h produced mature hybrids

Thus *N. alata,* used as female, gave hybrids with *Langsdorffii* and *Sanderae*; but when pollinated by other species it dropped its flowers. When used as male, however, *alata* produced hybrids not only with *Langsdorffii,* but also with *longiflora, plumbaginifolia,* and *Tabacum. Langsdorffii* as female shows the same results as *alata,* but as male exhibits some differences.

Trigonophylla, which is placed by DUNAL (1852) in the *Rustica* and by COMES in the Petunioides section, produced defective seedlings when pollinated by *Langsdorffii* of the Rustica section, and with *alata* and *Sanderae* of the Petunioides section it did not even give viable seeds. *Glutinosa* which is in the Rustica section, gave the same results when pollinated with these species. In general, the species with 12 haploid chromosomes show a very weak power of hybridization, *acuminata* and *trigonophylla* producing no hybrids with any of the species used in these experiments.

In species with 24 chromosomes the hybridizing ability is somewhat greater than in those with lower chromosome numbers, but compatibility between species shows no significant relation to taxonomic status.

Out of 186 crosses 84 were made reciprocally, and 67 of these crosses were between species differing in their chromosome number. In 56 of the latter no mature hybrids were obtained when the species with the lower chromosome number was used as female, but a higher degree of compatibility was shown when the species with the higher chromosome number was used as female. In 8 crosses where the species with the lower chromosome number was used as female the pollinated flowers dropped, while from the reciprocals mature hybrids were raised. In 4 other crosses where the species with the lower chromosome number was used as female, defective seeds were produced; while in the reciprocals mature hybrids were obtained. In 13 crosses the pollinated flowers dropped from the species with the lower chromosome number, while the reciprocals produced defective seedlings. In 19 crosses the pollinated flowers of the species with the lower chromosome number also dropped, although the reciprocals produced defective seeds. In one cross both types of crossing resulted in defective seedlings, and in another only defective seeds were produced. In 9 crosses the incompatibility between the species was so great that the pollinated flowers dropped in both the direct and reciprocal crosses. In one cross defective seeds were produced by using the species with the smaller number of chromosomes as female, and defective seedlings by the reciprocal.

In 8 reciprocal crosses the degree of compatibility appeared to be greater when the species with the lower chromosome number was used as female, but none of these crosses produced mature hybrids. In 6 of these crosses the flowers of the species with the lower chromosome number produced defective seeds, while the flowers of the reciprocals dropped after pollination. In 2 crosses defective seedlings were produced when the species with the lower chromosome number was used as female; in one of the reciprocals defective seeds were produced, in the other the flowers dropped.

Mature hybrids were obtained in both directions from only three of the 67 crosses involving species differing in their chromosome number, but in two of them (*glutinosa* × *Bigelovii* and *sylvestris* × *Tabacum*) a much larger number of plants per capsule were obtained when the female parent had the higher chromosome number.

Mature hybrids were obtained in both directions from only two (*alata* × *Langsdorffii* and *longiflora* × *plumbaginifolia*) of the 17 reciprocal crosses between species having the same chromosome number. Of 10 reciprocally made crosses between species with 12 haploid chromosomes, 4 did not produce seeds, no matter which parent was used as female. In 3 crosses the flowers dropped after pollination, while their reciprocals produced defective seeds. In 1 cross defective seeds were produced by both parents. From 2 crosses (*glutinosa* × *sylvestris* and *paniculata* × *glutinsoa*) a few very weak mature hybrids were raised, although their reciprocals produced respectively defective seeds and nothing.

Out of 5 reciprocally made crosses between species with 24 haploid chromosomes, 2 produced very vigorous hybrids, while their reciprocals produced defective seedlings and nothing. In a third cross defective seedlings were produced in one direction and defective seeds in the other. In the fourth cross both female parents produced defective seeds. In the last cross the seeds were defective in one female parent and the flowers dropped in the other.

DESCRIPTION OF THE SPECIES HYBRIDS

A. Species hybrids which did not reach maturity.

Of the various crosses made, 19 produced hybrids which died in early seedling stages.

a. Hybrids obtained by crossing species having 12 with species having 8 as the haploid chromosome numbers are:

1. *N. trigonophylla* × *Langsdorffii*
2. *N. glutinosa* × *Langsdorffii*
3. *N. paniculata* × *alata*.

b. Hybrids obtained by crossing species having 12 and 10 as the haploid chromosome numbers are:

4. *N. paniculata* × *longiflora*
5. *N. paniculata* × *plumbaginifolia*
6. *N. plumbaginifolia* × *paniculata*.

c. Hybrids obtained by crossing species having 16 and 8 as the haploid chromosome numbers are:

7. *N. suaveolens* × *alata*.

d. Hybrids obtained by crossing species having 12 and 16 as the haploid chromosome numbers are:

8. *N. paniculata* × *suaveolens*.

e. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers are:
   9. *N. Bigelovii*×*Langsdorffii*
   10. *N. Tabacum* var. *macrophylla*×*Langsdorffii.*

f. Hybrids obtained by crossing species having 24 and 10 as the haploid chromosome numbers are:
   11. *N. Bigelovii*×*longiflora*
   12. *N. Bigelovii*×*plumbaginifolia*
   13. *N. Tabacum* var. *macrophylla*×*longiflora*
   14. *N. Tabacum* var. *macrophylla*×*plumbaginifolia.*

g. Hybrids obtained by crossing species having 24 and 12 as the . haploid chromosome numbers are:
   15. *N. Bigelovii*×*sylvestris*
   16. *N. Bigelovii*×*paniculata.*

h. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers are:
   17. *N. rustica*×*suaveolens*
   18. *N. suaveolens*×*Tabacum* var. *macrophylla.*

i. Hybrids obtained by crossing species having 24 and 24 as the haploid chromosome numbers are:
   19. *N. nudicaulis*×*Tabacum* var. *macrophylla.*

Of these crosses, only *N. suaveolens*×*Tabacum* var. *macrophylla* (germination 82 percent), *N. Tabacum* var. *macrophylla*×*plumbaginifolia* (germination 3 percent), and *N. nudicaulis*×*Tabacum* var. *macrophylla* (germination 16 percent) yielded a great number of seedlings. In all other crosses the germination was very low. In the cross *N. Tabacum* var. *macrophylla*×*plumbaginifolia*, the seedlings all died after developing the fourth or fifth true leaf. In most of the crosses the seedlings died before developing the second true leaf.

B. Species hybrids which reached maturity.
   a. Hybrids obtained by crossing species having 8 as the haploid chromosome number.

I. *N. alata*×*Sanderae* (Cl_{10})

The germination of the seeds obtained was 81 percent. The ten hybrids raised were sterile. They were vigorous but variable, which may indicate that *Sanderae* is heterozygous for various genes, since in other species crosses where *Sanderae* was used the hybrids were also variable.

Nearly all of the hybrids resembled *N. Sanderae* in habit of growth. The leaves were an intermixture of the characteristics of both parents. In corolla color the *Sanderae* parent was dominant; but in size and shape the flowers were nearly intermediate.

II. *N. alata* × *Langsdorffii*

The germination of the seeds obtained was 80 percent (93 percent in the reciprocal). The ten hybrids raised were fertile, vigorous, uniform, and in habit of growth resembled *Langsdorffii*. The leaves were nearly intermediate in color, size and shape. The flowers also were intermediate in color, size and shape. The pollen-grains were intermediate in color—slightly bluish.

III. *N. Langsdorffii* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 62 percent. The ten hybrids raised were fertile, vigorous, and variable in habit of growth, though resembling *Langsdorffii*. The leaves were different in size and shape in the different individuals. In respect to corolla color, *Sanderae* was dominant. In size and shape the flowers were different in the different individuals. The pollen-grains were intermediate in color—light bluish.

b. Hybrids obtained by crossing species having 10 and 8 as the haploid chromosome numbers.

IV. *N. longiflora* × *alata*

The germination of the seeds obtained was 95 percent. The fifty hybrids raised were sterile. They developed slowly into vigorous, uniform plants, in habit of growth an intermixture of the characteristics of both parents. The leaves were slightly rugose, like those of *longiflora*. The flowers, which remained slightly closed during the day, were intermediate in size and shape, though with the corolla lobes bluish externally as in *longiflora*.

V. *N. longiflora* × *Sanderae*

I was unsuccessful in this cross; but ten hybrids were raised from seeds obtained by DR. A. J. MANGELSDORF. The plants were sterile; and though variable in size and in habit of growth, were quite vigorous. The leaves of each individual were slightly rugose like those of *longiflora*. The flowers, although somewhat variable, resembled the two parents in size and shape. They remained slightly closed during the day like those of *longiflora*.

VI. *N. plumbaginifolia*×*alata*

The germination of the seeds obtained was 49 percent. The fifty hybrids raised were sterile. They developed slowly to vigorous, uniform plants, in habit of growth an intermixture of the traits of both parents. The leaves were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, bluish externally like *plumbaginifolia*, and remained slightly closed during the day.

VII. *N. plumbaginifolia*×*Langsdorffii*

The germination of the seeds obtained was 35 percent. The thirty hybrids raised were sterile. They were vigorous, uniform, and in habit of growth intermediate between the parents. The leaves resembled *Langsdorffii* in color, but were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, shape and color. The corolla lobes were yellowish on the upper side, but slightly bluish underneath. The pollen-grains were intermediate in color—slightly bluish.

c. Hybrids obtained by crossing species both having 10 as the haploid chromosome number.

VIII. *N. longiflora*×*plumbaginifolia*

The germination of the seeds obtained was 96 percent (81 percent in the reciprocal). The ten hybrids raised were fertile, very vigorous, uniform, and in habit of growth intermediate between the parents. The leaves and the flowers were also intermediate in their characteristics.

d. Hybrids obtained by crossing species having 12 and 8 as the haploid chromosome numbers.

IX. *N. paniculata*×*Sanderae* (Cl_{10})

The germination of the seeds obtained was 28 percent. The ten hybrids raised were sterile. They varied markedly in vigor, from very weak to very vigorous; they also varied in habit of growth. The leaves were variable in the different individuals, but were nearly all petiolate as in *paniculata*. The flowers in all were as red as those of the *Sanderae* parent, varying in size but more closely resembling *paniculata* in shape.

X. *N. paniculata*×*Langsdorffii*

The germination of the seeds obtained was 12 percent. The ten hybrids raised were sterile. The plants were uniformly very weak, in habit of growth resembling *Langsdorffii*. The petiolate leaves resembled *paniculata* rather closely. The flowers in size and shape resembled *Langsdorffii*. The pollen-grains were intermediate in color—slightly bluish.

e. Hybrids obtained by crossing species both having 12 as the haploid chromosome number.

XI. *N. paniculata*×*glutinosa*

The germination of the seeds obtained was less than 1 percent. The five hybrids raised were sterile. The plants were very weak at the beginning, but quite vigorous at the end. They exhibited little variablity, and were an intermixture of the characteristics of both parents. The first leaves were oblong, the later ones cordate, thus resembling *glutinosa*. The flowers were yellow, tinged with pink, and were intermediate in size and shape.

XII. *N. glutinosa*×*sylvestris*

The germination of the seeds obtained was 5 percent. The two hybrids raised were sterile. They developed slowly and were very weak, though uniform and in habit of growth intermediate between the parents. The leaves were nearly petiolate as those of *glutinosa*, but oblong like those of *sylvestris*. The flowers were pink as in *glutinosa*, but in size and shape intermediate.

f. Hybrids obtained by crossing species having 16 and 10 as the haploid chromosome numbers.

XIII. *N. suaveolens*×*longiflora*

The germination of the seeds obtained was 22 percent. The thirty hybrids raised, though sterile, were very vigorous and in habit of growth resembled *suaveolens*. The leaves also resembled *suaveolens*. The flowers were more like *suaveolens* in size and shape; but in corolla color *longiflora* was dominant, the corolla lobes being bluish underneath.

XIV. *N. suaveolens*×*plumbaginifolia*

The germination of the seeds obtained was 2 percent. The fifteen hybrids raised, though sterile, were very vigorous, uniform, and in habit of growth resembled *suaveolens*. The leaves and flowers resembled *suaveolens*, though in corolla color *plumbaginifolia* was dominant, the corolla lobes being bluish underneath.

g. Hybrids obtained by crossing species having 16 and 12 as the haploid chromosome numbers.

XV. *N. suaveolens* × *glutinosa*

The germination of the seeds obtained was 88 percent. The five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *suaveolens*. In size and shape the leaves were intermediate, but petiolate as those of *glutinosa*. The flowers were pink like those of *glutinosa*, but in size and shape resembled *suaveolens*.

h. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers.

XVI. *N. Tabacum, Cuba* × *alata*

The germination of the seeds obtained was less than 1 percent. The two similar hybrids raised were sterile, very weak, and in habit of growth resembled *Tabacum*. The characteristics of both leaves and flowers were more nearly like those of *Tabacum*.

XVII. *N. Tabacum*, white tobacco × *Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. Three hybrids were raised from a cross in which *Sanderae* Cl_{10} was used. All of them were sterile, quite weak, and though different, were more nearly like *Tabacum*. The leaves and the flowers resembled *Tabacum* in size and shape, but the corolla color of *Sanderae* was dominant. From another cross, where *Sanderae* N16 with bluish corolla was used, the four hybrids obtained showed the result of segregation in corolla color, three being bluish and one white. These hybrids also were sterile, weak, and variable in habit, though resembling *Tabacum*.

XVIIII. *N. rustica* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. The four hybrids raised were sterile. The plants varied markedly in vigor and size, although in habit of growth they resembled *rustica*. The leaves of all were petiolate, and in size and shape were like those of *rustica*. The flowers although different in size and shape in the different individuals, resembled *rustica*, except that the red corolla color of *Sanderae* was dominant.

XIX. *N. rustica* × *Langsdorffii*

The germination of the seeds obtained was less than 1 percent. The only hybrid raised was sterile, very weak and in habit of growth like *rustica*. The leaves were petiolate, resembling *rustica*, in size and shape. The flowers also resembled *rustica* in size and shape. The pollen grains were intermediate in color—slightly bluish.

i. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers.

XX. *N. nudicaulis* × *trigonophylla*

The germination of the seeds obtained was 46 percent. The twenty hybrids raised were sterile, though quite vigorous, uniform and with a habit of growth like *nudicaulis*. The leaves were also more like those of *nudicaulis*. The corolla limb was curved as in *nudicaulis*, but the tube intermediate between those of the parents in length.

XXI. *N. glutinosa* × *nudicaulis*

The germination of the seeds obtained was 10 percent. The twenty-eight hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the characteristics of both parents. In color, the leaves were like those of *nudicaulis*, but petiolate as in *glutinosa*. The flowers were like those of *glutinosa* in color and shape, and like those of *nudicaulis* in size.

XXII. *N. Bigelovii* × *glutinosa*

The germination of the seeds obtained was 98 percent, but less than 1 percent in the reciprocal. The fifty hybrids raised were sterile, vigorous, uniform and in habit of growth resembling *Bigelovii*. The leaves were petiolate like those of *glutinosa*, but in size, shape and color resembled *Bigelovii*. The flowers were slightly pink, but in size and shape resembled *Bigelovii*.

XXIII. *N. Tabacum* var. *macrophylla* × *sylvestris*

The germination of the seeds obtained was 50 percent. The seventy-five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Tabacum*. The leaves and the flowers resembled *Tabacum*, but the corolla tube was more slender, the corolla limb smaller and the corolla lobes narrower than those of *Tabacum*. The reciprocal cross was made by using another *Tabacum* variety as the male parent. Here the germination of the seeds obtained was less than 1 percent. The three hybrids raised behaved in respect to the parental characters as in the cross *macrophylla* × *sylvestris*.

XXIV. *N. Tabacum* var. *macrophylla*×*glutinosa*

The germination of the seeds obtained was 25 percent. The twelve hybrids raised were sterile, very weak, variable in size and habit, an intermixture of the traits of both parents. The leaves were petiolate, small and ovate, more like those of *glutinosa*. The flowers were as red as those of *macrophylla*, but in size and shape were intermediate.

XXV. *N. rustica*×*paniculata*

The germination of the seeds obtained was 35 percent. The fifteen hybrids raised were partially fertile, quite vigorous, uniform and in habit of growth resembled *rustica*. The leaves and the flowers approached closely to those of *rustica*, but the corolla tube was somewhat intermediate in length.

j. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers.

XXVI. *N. Bigelovii*×*suaveolens*

The germination of the seeds obtained was 84 percent (98 percent in the reciprocal). The thirty-three hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Bigelovii*. The leaves and flowers were more like those found in *Bigelovii*.

k. Hybrids obtained by crossing species having 24 as the haploid chromosome number.

XXVII. *N. Bigelovii*×*nudicaulis*

The germination of the seeds obtained was 15 percent. The twenty hybrids raised were sterile, vigorous, uniform and in habit of growth an intermixture of the traits of both parents. The leaves resembled *Bigelovii*. The flowers were intermediate in color, but were small with curved corolla as in *nudicaulis*.

XXVIII. *N. Bigelovii*×*N. Tabacum* var. *macrophylla*

The germination of the seeds obtained was 15 percent. The twenty-five hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the traits of both parents. The leaves and flowers were also intermediate.

XXIX. *N. rustica*×*N. Tabacum* white tobacco

The germination of the seeds obtained was less than 1 percent. The two hybrids raised were slightly fertile, vigorous, and in habit of growth intermediate. The leaves were petiolate, and in shape were more nearly like *rustica*; but were intermediate for size. The flowers were intermediate in color, size and shape.

CYTOLOGICAL OBSERVATIONS ON CERTAIN NICOTIANA SPECIES HYBRIDS

A. *N. alata* × *Langsdorffii*

The heterotypic and homeotypic divisions in the pollen mother cells are normal.

B. *N. longiflora* × *Sanderae*

The reduction division shows a picture very similar to that of the Drosera hybrids (Rosenberg 1909). There are eight bivalents and two univalents. Sometimes it appears that in an earlier stage of the heterotypic metaphase the univalents are in the same plane as the bivalents, but frequently one observes spindles with two lagging chromosomes (figure 37). During the second metaphase (figure 38), two univalent chromosomes regularly lag outside both equatorial plates. In figure 39, one of the univalents has already split. During the first and second divisions the univalents are usually distributed at random to the poles in a normal manner, but cases in which the 4 univalent chromosomes are lagging (figure 40) are frequently observed.

C. *N. Tabacum* × *alata*

The reduction division here is quite irregular. In the first division as many as 8 gemini can be distinguished (figure 41 and 43). Figure 41 shows the chromosomal arrangement at the multipolar spindle immediately after diakinesis. Figure 42 shows the next stage with the chromosomes aligned on the equatorial plate. Such figures are rather infrequent, however, for soon after this stage, the univalents pass to the poles. The first metaphase spindle most commonly seen is shown in figure 43. Eight bivalents and three univalents lie in the equatorial plate, the rest of the univalents being on the way to the poles. It seems that soon after some of the univalents have reached the poles, the bivalents begin to divide (figure 44). Figure 45 represents a situation occasionally occurring in which there has been precocious division of some of the chromosomes either during the final stages of the first division or at the beginning of the second division, the chromosomes of both plates plus the lagging one are $18+19+1=38$ instead of 32. Presumably 6 chromosomes have already divided. Such chromosomes, that is to say those which have gone through the equational division at the end of the first division, seem to behave during the second anaphase much as do those of the Triticum hybrids (SAX 1922, KIHARA 1924, THOMSON 1926). I think that the fairly large number of lagging chromosomes found during the second division (figure 46) are not merely descendants of the few chromosomes which lagged during the first division, but also of those which have not undergone the equational division at the same time as the majority.

D. *N. Tabacum* × *Sanderae* (Cl_{10})

The reduction division in these hybrids is similar to that found in the hybrids of *N. Tabacum* × *alata*. Diads were frequently found.

E. *N. paniculata* × *Langsdorffii*

No bivalent chromosomes were observed during the various stages from early prophase to the second division. In figure 47, a heterotypic spindle, four of the chromosomes are very long and slender; but it is clear from the preparation that they are attached at both ends to spindle fibers. In a slightly later stage (figure 48), two such chromosomes are shown so securely fastened to a fiber that they seem to be pulled apart. At the end of the first division the chromosomes are regularly distributed between both poles, and during the second metaphase the number of lagging chromosomes is usually small (figure 49). An exceptional case is illustrated in figure 50 where all chromosomes lie in the same plane. The second division is virtually an ordinary equational division (figure 51).

F. *N. rustica* × *paniculata*

In the heterotypic metaphase of this hybrid the 12 bivalent chromosomes are easily distinguished from the 12 univalents. The bivalents are about twice as large and somewhat longer than the univalents, and are always found on the equatorial plate. The spherical univalents are scattered. Figure 52 shows the 12 bivalents in the center, 5 univalents on one side, 6 univalents on the other side, and one univalent overlapping one of the bivalents. The group of 5 lies in one plane, the group of 6 in another plane, while the equalorial plate with the 12 bivalents lies between them.

The arrangement of the chromosomes during the heterotypic anaphase is illustrated by figure 53. The bivalents, which divide soon after the univalents approach the poles, are found together with the univalents near the poles. Figures 54 and 55 show two sets in the second metaphase. In the first figure the chromosome number of the two plates is $18+18=36$, in the second $16+20=36$. This indicates that no univalent chromosomes had split during the first division. This is the case for all counts made at this stage. (Cases of lagging chromosomes were also found). The second division is usually very regular (figure 56) though figures with lagging chromosomes have occasionally been observed.

G. *N. suaveolens*×*glutinosa*

During the first metaphase the chromosomes of both parents, 16+12=28, are scattered throughout the spindle, with no bivalents visible (figure 58). At the heterotypic anaphase the chromosomes are distributed at random to the two poles, lagging chromosomes being quite common. Figure 57 illustrates a second metaphase with 9 chromosomes in one equatorial plate and 17 in the other, while outside there are 2 lagging chromosomes. Figure 59, of the same stage, shows 4 chromosomes on a small spindle, 2 chromosomes outside, and 22 chromosomes on the equatorial plate. The chromosomal distribution during this stage is quite variable. The second division is chiefly equational. The chromosomes which lag during this division seem to be descendants of univalent chromosomes which, at the beginning of the second division, failed to reach the equatorial plates. The tetrads show a great deal of degeneration. Very often, in acetocarmine preparations, they stuck together, appeared to be shrunken, and failed to stain.

H. *N. suaveolens*×*Bigelovii*

During the heterotypic division it is very difficult to distinguish the metaphase from the anaphase, nevertheless it is easy to see that the chromosomes do not pair. The chromosomes of both parents (16+24) are scattered along the spindle. Figure 60a-b shows 37 chromosomes lying in one section and three in the other. Because the chromosome distribution in the first division is so variable the second metaphase looks quite different in different cells. Figure 61 shows 21 chromosomes lying on one plate, 16 on the other plate, and 3 lagging. Figure 62 shows the same stage in which the chromosomes are so distributed that 38 chromosomes lie on one plate, while 2 are arranged as a small spindle. The spindle fibers in this case are not visible, but the position of the chromosomes indicates a spindle. Figure 63 presents a second metaphase in which the chromosomes are distributed among three very well formed spindles, with 2 chromosomes lagging in a spindle position. The second division is regular. At this stage the chromosomes of the different spindles normally undergo the equational split simultaneously, and any lagging chromosomes which are found during and after the second division are presumably descendants of the lagging chromosomes seen during the first division. In figure 64, showing the end of the second division, there are 8 nuclei visible. In three of the small nuclei there are 6 chromosomes; in another nucleus there are 5 chromosomes; in two other nuclei there are 10 chromosomes; while in the two very large nuclei there are 19 and 13 (?) chromosomes respectively. It is very difficult to count accurately in such a stage since the prochromosomes lie in so many different planes. I am inclined to believe that in this case, however, both of the very large nuclei really contain 19 chromosomes, the two next largest contain 10, two of the smaller ones have 6, and the other two only 5 chromosomes, originating through a chromosome distribution during the first division in which the formation of four spindles containing $19+10+6+5=40$ chromosomes took place at the beginning of the second division. The degeneration begins at the second telophase. The protoplasm is almost always very poorly fixed, the cross walls being shrunken and the nucleus itself taking the stain irregularly. It may be that the disperison of non-homogeneous chromatin between so many nuclei is due to the acceleration of the degeneration.

I. *N. rustica* × *Tabacum*, white tobacco

The first reduction division is very irregular. Although I examined many slides showing the heterotypic division there is so much variation that I could not be certain whether gemini were formed or not. In the two serial sections of figure 65 a and b, a very common situation is illustrated in which the 48 chromosomes scattered on the spindle can be easily distinguished. In some cases I have been unable to count 48 chromosomes, which I assume to be due to the presence of loose gemini.

During the second metaphase the chromosomes are distributed irregularly to both poles, some of them lagging on either side of the equatorial plates. In figure 67 is illustrated the most unequal distribution found; 36 chromosomes in one plate, 11 in the other plate, and 1 chromosome lagging between them. In anthers showing different stages of the second division, especially of the second metaphase, fairly normal single spindles were visible which were twice as large as ordinary second division spindles. Similar cases are reported by Miss LJUNGDHAL (1922) in the cross *Papaverum somniferum* × *orientale*. Such cases were thought by her to have originated through the fusion of two second metaphase spindles. Figure 66 corresponds roughly to figure 5d of her plates, though the two cases are somewhat unlike because of the different type of division. Figure 68 illustrates a case in which a cell wall has already developed between the two spindles. It is not impossible that the peculiar chromosome distribution caused the development of the cell wall thus halting the second division just as it was starting, the result being the formation of the diads frequently found in the pollen mother cells of this hybrid. Normally the second division is very irregular, with many lagging chromosomes scattered over the two spindles. In figure 69a,b,c, the chromosomes lying in the plane of one spindle are represented in solid black, those of the other spindle in stipple, the lagging chromosomes in outline.

DISCUSSION

Inheritance in species hybrids

The gradual growth of the chromosome theory of heredity has led geneticists to interpret the anomalous distribution of characters in certain species crosses to an abnormal distribution of the chromosomes. Where the hybrids are fertile and the chromosome behavior is that which is ordinarily found in varietal crosses, the normal type of inheritance obtains (*Vide Mirabilis jalapa*×*M. longiflora,* CORRENS 1909; *Antirrhinum majus*×*A. molle,* BAUR 1911, LOTSY 1912; *Nicotiana Langsdorffii*×*N. alata,* EAST 1916). In a few cases, even partially fertile hybrids have shown a behavior similar to that found in intervarietal crosses (*Godetia Whitneyi*×*G. amœna,* RASMUSSON 1920). In general, however, one cannot go very far in a pedigree culture analysis of the results of species hybridization; though it appears probable, following STURTEVANT's work on *Drosophila melanogaster* and *D. simulans* (1920, 1921) that related species may have genes in common and may produce similar mutants.

Lack of sufficient analysis in intervarietal crosses prevents any examination of Nicotiana species hybrids after the manner used for Drosophila. SACHS-SKALINSKA (1921), however, has demonstrated Mendelian segregation for corolla color in the fertile hybrid, *N. Langsdorffii*×*Sanderae.* The colored corolla of *N. Tabacum* var. *macrophylla* which is dominant in intervarietal crosses with white tobacco has also proved dominant in the cross with *N. sylvestris,* and in somewhat diluted form in the cross with *N. Bigelovii.* The red corolla color of a *N. Sanderae* variety proved dominant over the white of *N. alata* and white tobacco. This corolla color of *N. Sanderae* was also dominant over the yellow-greenish corolla color of *N. Langsdorffii, N. paniculata,* and *N. rustica.* An intermediate corolla color occurred in the hybrid *N. Bigelovii*×*glutinosa* as well as in *N. Tabacum* var. *Cuba*×*alata* and *Cuba*× white tobacco. In intervarietal crosses of *Tabacum* it has been found (SETCHELL, GOODSPEED, and CLAUSEN 1922) that the petioled leaf shape is more or less dominant over the sessile. Similarly, the petioled leaf character of *N. rustica*, *N. paniculata*, and *N. glutinosa* proved dominant in the various interspecific crosses involving these species.

The various hybrids derived by crossing species having the same chromosome number show a mixture of the parental characters due, presumably, to lack of dominance or to a similar number of dominants contributed by each parent. Where, on the other hand, the hybrids are obtained by crossing species widely differing in their chromosome number, as *N. Tabacum*×*Sanderae*, *N. Tabacum*× *sylvestris*, *N. rustica*×*Langsdorffii*, *N. suaveolens*×*longiflora*, *N. suaveolens*×*plumbaginifolia*, the hybrid resembles the parent having the larger chromosome number except in the cross *N. paniculata*×*Langsdorffii* in which the latter species appears to be dominant apart from the petioled condition of the leaves. It is not impossible that this resemblance to *N. Langsdorffii* is due to a few striking dominant characters contributed by it.

The characteristics of the sterile hybrid *N. Tabacum*×*sylvestris*, in which *N. Tabacum* is better represented, led GOODSPEED and CLAUSEN (1916) to conclude that distinct reaction systems are involved in species crosses, and that the phenomenon must be viewed in the light of a contrast between systems rather than between specific factor differences. Although they have, in some instances, recognized the influence of *N. sylvestris* on the hybrid, these authors have questioned the plate in BAUR's textbook in order to establish the complete dominance of *N. Tabacum.* KLEBS (1917), however, claims an intermediate character for the flower of this hybrid, and the intermediate corolla length of the hybrid can be distinguished in plates given by GOODSPEED and CLAUSEN (1917). Although the calcyina flower is almost completely recessive in intervarietal *Tabacum* crosses (SETSHELL, GOODSPEED, and CLAUSEN 1922), it appears in the *N. Tabacum*×*sylvestris* hybrid, albeit in not so extreme a form as in the parental types. The probability is that the twelve chromosomes of *sylvestris* do not carry the factors necessary to limit the appearance of this character, and that in general, the great resemblance to *Tabacum* is due to the transmission to the hybrid of a larger number of dominant factors with the larger number of chromosomes. The high degree of sterility, on the other hand, depends (EAST 1915, 1921) on the interrelation between the chromosomes.

A species hybrid parallel to that cited by Goodspeed and Clausen is found in crosses between white tobacco and two different *N. Sanderae* plants. The hybrids obtained show a high degree of resemblance to the *N. Tabacum* parent, but corolla color of the *Sanderae* parent is dominant.

Non-viability of the gametes in species hybrids

In the matter of sterility through the production of non-viable gametes, it is necessary to distinguish between completely sterile and partially sterile hybrids. The first category is the one most frequently found in *Nicotiana*; the second I have found only in *N. rustica*×*paniculata*. From cytological studies of different hybrids of the first category, such as *N. Bigelovii*×*suaveolens*, *N. suaveolens*×*glutinosa*, and *N. paniculata*×*Langsdorffii*, complete sterility seems to be the logical expection, since no pairing of chromosomes occurs during the first division. The chromosomes are distributed irregularly between the two poles of the heterotypic spindle, some being left behind; or, as in *N. Bigelovii*×*suaveolens*, they are distributed among three or four homeotypic spindles. In *N. rustica*×*paniculata*, on the other hand, there is distinct pairing of the chromosomes. Presumably this should afford a greater expectation of the production of viable gametes, though there are cases known where a seemingly normal reduction does not lead to viable gametes.

Of some interest is the relation between the chromosome behavior and the morphological appearance of the hybrids. *N. paniculata* and *N. Langsdorffii* both belong to the *rustica* section, but they produced very weak hybrids with no pairing of chromosomes. It seems that the mode of the reduction division is not correlated with the vigor of the hybrid, nor even with the relationship between the species, although this case is somewhat questionable as the accepted taxonomic position of *Langsdorffii* is open to criticism (Lock 1909). *N. suaveolens* and *Bigelovii*, both of the *Petunioides* section, must have some degree of genetic compatibility as demonstrated by the extreme vigor of their hybrids, but again there was no pairing of chromosomes in the reduction division, In the hybrid *N. suaveolens*×*glutinosa* I again found hybrid vigor and non-pairing of chromosomes, but here the parents are not closely related taxonomically.

It is to be noted that regularly in species hybrids of Triticum and other genera, the longitudinal split of the univalent chromosomes occurs during the first division (Kihara 1924, Sax 1922, Thomson 1926); but in Nicotiana in all the species hybrids studied no longitudinal split of the univalent chromosomes occurred during the first metaphase.

Non-viability of the zygote in species hybrids

Sterility in plant and animal hybrids is sometimes due to the fact that the zygote dies during its development. Such cases are found very frequently in Drosophila, where they are due to definite lethal or semi-lethal factors (BRIDGES, MORGAN and STURTEVANT 1925). I have observed some interesting cases of zygote mortality in certain Nicotiana hybrids, such as *N. suaveolens* × *Tabacum* var. *macrophylla*. The cross is easily made and the germination of the seeds is nearly normal. After germination the seedlings start their growth at a rapid rate with a tendency to hybrid vigor until the first true leaves appear. At this stage the seedling growth stops, and after a time the plants die. This phenomenon was confirmed by three or four successive sowings. The leaves of the seedlings were a normal green as if no disturbance had occurred in the chlorophyll development. The root system, however, which was quite normal at the beginning, began to degenerate rather early although the leaves remained green a long time after. This was characteristic of all hybrids which died during vegetative development. This same cross was successful for SAGERET and for GÄRTNER, and their plants reached maturity. It is rather peculiar that in the reciprocal cross the pollinated *Tabacum* flowers dropped soon after pollination, although crosses were almost always successful when using species with the higher chromosome number as female.

The hybrid *N. Tabacum* var. *macrophylla* × *glutinosa* stopped its growth in a later stage, after from 5 to 10 leaves had developed. The root system which was very well developed degenerated with the cessation of growth, but long after that the plant was still green. I was inclined to believe that some pathological organism was responsible for the death of the roots, although the neighboring plants of other crosses were normal. I cut away the old root of several plants, disinfected them in Uspulun, and grew them in sterilized soil under artificial light 3–4 hours during the night, as the day was very short at that time. The plants recovered, and after only one week I was able to see a new root developing normally. New leaves were formed, and in three weeks the plants came into flower. Sister plants growing without artificial light never came to flower. It seems that for some reason metabolism was abnormal, and that this defect was partially nullified by increased exposure to light. This success encouraged me to try defective plants of hybrid combinations under similar conditions, and I was especially hopeful of the hybrid *N. Tabacum* var. *macrophylla* × *plumbaginifola*; but had no success. Thirty plants from two sowings were observed. In both cases the seedlings started with a very rapid growth, but stopped after the 3d or 4th leaf developed.

The single seedlings obtained from *N. Tabacum* var. *macrophylla* × *Langsdorffii* and from *N. Tabacum* var. *macrophylla* × *longiflora* had deformed cotyledons and no visible growing point. The same *N. paniculata* plant was pollinated by three different *alata* plants and in two of the crosses the seedlings died after developing the fourth true leaf. In this case I am not sure whether they died because of their genetic constitution or because of the greenhouse conditions during the winter, or, perhaps, a combination of both. One plant of *N. Tabacum* var *Cuba* was pollinated by two different *alata* plants and by a *Sanderae* plant. With one of them, *alata*, No. 20, three capsules were obtained, but not a single seed germinated. With *alata*, No. 25, the seedlings produced died in different stages during their development and only two finally reached maturity. With *Sanderae*, No. 16, four seedlings reached maturity. The chromosomes were counted in the root-tips of some of the hybrids seedlings of *cuba* × *alata* No. 25, which died after developing the fourth true leaf and the number was found to be over 30.

From the cross *N. Tabacum* var. *macrophylla* × *nudicaulis* I obtained plump seeds having endosperms, but which did not germinate. I do not know whether the seeds contained embryos or not, as I did not make a histological investigation.

Incompatibility between species in Nicotiana

The degree of compatibility between different species is supposed to be indicated by their taxonomic relationship. This study has shown that, with some exceptions, hybridization is successful more frequently when the species with the higher chromosome number is used as female. While these results are not entirely in accord with those of other investigators, I believe that this correlation holds in Nicotiana. The literature of genetics affords no clearly parallel case. From the hybridization results between the various Triticum species as reported by different investigators, it is not clear in which direction the cross is easier, but it is evident that the species with the lower chromosome number is generally used as the female parent. Of the 26 crosses reported (TSCHERMAK 1914, SAX 1922, KIHARA 1924) in only 4 is the species with the higher chromosome number used as the female. KIHARA reports successful pollination of a species in the Emmer group ($n=14$) by one of the *monococcum* group ($n=7$). The other three cases are reported by SAX who successfully pollinated three species of the *vulgare* group ($n=21$) by three of the Emmer group. Similar results have been reported for Papaverum species crosses, where 4 out of 17 hybrids (FOCKE 1881, LJUNGDAHL 1924) were produced by using pollen of species with the higher chromosome number. Unfortunately, these investigations do not make clear just what crosses were attempted, and which were successful.

CONCLUSION

The chromosome numbers of the different Triticum species (SAKAMURA 1918, SAX 1918) have been found to be what should be expected from the taxonomic arrangement of the species, the interspecific hybridization results, the serological studies, and resistance to parasites. The three sections of the genus are represented by three different numbers, each found only among the species of a given section, and the three numbers establish the polyploid series 2x, 4x, 6x, with the basic number 7. In Fragaria (LONGLEY 1926, ICHIJIMA 1927) the situation is similar. In Nicotiana, however, as in Chrysanthemum (TAHARA 1921), and Rosa (TÄCKHOLM 1922), each taxonomic section contains more than one characteristic chromosome number. Species with an aberrant number of chromosomes in a polyploid series, as in *longiflora* and *plumbaginiolia* in Nicotiana are frequently observed in various genera. KUWADA (1919) assumed for maize that transverse segmentation of some of the chromosomes is a genetically fixed phenomenon. HEILBORN (1924), however, believes that in Carex the higher chromosome number in various related species arose practically always through non-disjunction of single whole chromosomes rather than through fragmentation; and that hybridization was an important factor in the perpetuation of the new chromosome number. If transverse division is to be taken as the explanation of the origin of some Nicotiana chromosome numbers, one may explain the 10 haploid chromosomes of certain species as arising by the occasional transverse division of two long U-shaped chromosomes, and the 12 chromosome type by the transverse division of four long U-shaped chromosomes of certain 8 chromosome species. It is to be noted, however, that the 12 chromosome species have a large number of U-shaped and very long chromosomes.

WINGE's (1917) theoretical interpretation of the origin of new forms by the reduplication of all the chromosomes of two species after hybridization is probably important in connection with Nicotiana species (CLAUSEN and GOODSPEED 1925). The morphological appearance of certain related Nicotiana species indicates that they have a common origin. *Rustica* and *paniculata* differ noticeably only in respect to corolla length and shape, for example. *Nudicaulis* and *trigonophylla* are also similar to each other, and also show the same chromosome relation, 24:12. The three species *glutinosa*, *tomentosa*, and *Rusbyi* show a gradation in the morphological characteristics of the two sections *Tabacum* and *Rustica*, *glutinosa* showing largely *Rustica* characters, *Rusbyi* largely *Tabacum* characters, with *tomentosa* standing in between. Such instances point to hybridization as a possible origination of species.

The possibility that the species have originated as multiples from a type having the basic number 4, might be indicated if the reduction division of any of the hybrids showed this number of bivalents among the univalents or *vice versa*, as has been found in Triticum (Sax, K., and Sax, H., 1924); but no such cytological situation has been found.

SUMMARY

1. Nineteen species of Nicotiana have been studied cytologically, and species with the following haploid chromosome numbers were found: 8, 10, 12, 16, 24. All of these numbers have occurred among the species belonging to the Petunioides section. Among the species of the *Rustica* section, the haploid numbers found were 8, 12, and 24. Differences in shape and size were found among the chromosomes of the different species and in certain cases among the chromosmes of a single species. Counts made in the root-tips confirmed the numbers found in the pollen-mother cells. There are great differences in size and shape between the chromosomes of the somatic cells and those of the pollen-mother cells, the first being very long, entwined, and generally U-shaped, while the latter spherical or bean-shaped.

2. Fifteen species were crossed with the view of discovering if any parallelism exists between their taxonomic relationship and their ability to hybridize. Four degrees of compatibility between the species were distinguished according to the results obtained in the hybridization experiments: (1) Crosses in which the flowers pollinated dropped after the pollination; (2) those in which capsules were produced without seeds, or with seeds which did not germinate; (3) those in which the seedlings died before reaching maturity, and finally (4) those which produced mature hybrids. It was found further that the species with 8 haploid chromosome numbers produced fertile hybrids (as did species with 10 haploid chromosomes); but the ability to hybridize was very low between the species with 12 chromosomes, though less difficult between the species with 24 chromosomes. In crosses between species differing in their chromosome number, success was rare when species with a lower chromosome number were used as female; and in the cases where the cross succeeded in both directions, the percentage of successes was less when the female parent had the lower chromosome number.

3. A morphological description of the species hybrids obtained is presented. In crosses between species with the same chromosome number, the fertile hybrids, generally speaking, were intermediate, but

the great variability of the F_2 plants and the obvious Mendelization of the striking qualitative differences leads one to believe that the genes are distributed as in ordinary varietal crosses. In hybrids between species differing in their chromosome number, the parental type with the greater number of chromosomes was better represented morphologically.

4. In nine of the species hybrids, the reduction division in the pollen-mother cells was studied. Four types of division were found. In *N. alata*×*Langsdorffii* (8×8), the reduction was normal, no univalents being found. In *N. longiflora*×*Sanderae* (10×8), *N. Tabacum Cuba*×*alata* (24×8), *N. Tabacum* white×*Sanderae* Cl_{10} (24×8), and *N. rustica*×*paniculata* (24×12), the number of bivalent chromosomes was equal to the haploid chromosome number of the parent with the lower chromosome number. In *N. rustica*×*N. Tabacum* white tobacco (24×24) a few loose pairs appeared to be present. In *N. paniculata*×*Langsdorffii* (12×8), *N. suaveolens*×*glutinosa* (16×12), and *N. suaveolens*×*Bigelovii* (16×24), no pairing of chromosomes occurred.

In cases in which a regular pairing of chromosomes was observed, the univalents were usually distributed to both poles without splitting during the heterotypic division, though occasionally lagging chromosomes were found. In cases where no pairing was observed, the chromosomes were distributed irregularly to the poles; and in some few cases, particularly in hybrids between species with high chromosome numbers the occurrence of three spindles during the second metaphase was observed. The reduction division of those hybrids in which some few loose pairs occurred during the heterotypic metaphase or before, was most complicated and irregular; and this type of irregularity was observed in a hybrid where the total number of chromosomes was high (24×24); here diads were observed among the tetrads.

The second division was always a regular equational division.

5. The bearing of these results on certain theoretical problems is discussed. Where the reduction division is normal, inheritance appears to be normal. Where the number of chromosomes contributed by the two parents is identical and the reduction division is abnormal, the hybrids show an intermixture of the characteristics of the two parents, presumably because of lack of dominance or because an approximately equal number of dominants is contributed by each parent. Where the number of chromosomes contributed by the two parents is unequal, the hybrids tended to resemble the parent with the higher chromosome number, with but few exceptions. In these exceptional cases, perhaps the con-

tribution of a few striking characters by the species having the lower chromosome number tends to distort the result.

6. The hybrids which reached maturity were completely sterile, with the exception of *N. rustica*×*paniculata*; and this hybrid was partially sterile. Non-viability of the gametes seems to parallel irregular reduction division.

7. The compatibility of the species shows a greater relation to the chromosome number than to the taxonomic status, but the mere fact that two species have the same chromosome numbers does not mean that they will cross. In general a cross can be made more easily when the species with the higher chromosome number is used as the female parent.

8. There is no particular correlation between the vigor of the hybrid and the mode of the reduction, except that in cases where the reduction division is normal, the plants are never weak. Cases were found in which the hybrid was weak although chromosome pairing occurred, as in *N. Tabacum*×*alata*. On the other hand, in *N. Bigelovii*×*suaveolens*, where no chromosome pairing occurred, the hybrid was very vigorous.

9. Weak hybrids can sometimes be aided in their development by a change in external conditions, such as an increase in the relative amount of light.

10. Though the Nicotiana species probably did not originate by multiplication of 4 chromosomes, it is possible that they did originate from one or more 8-chromosome species by a reduplication of certain chromosomes or of whole sets of chromosomes.

After this paper was completed, there was an opportunity for studying cytologically the following two species of the genus:-

N. caudigera Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised are large, branched, with long petioled leaves, cordate below and lanceolate above, having spine-like glandular hairs on the surface and on the margin. The corolla is white with broad cylindrical tube and short, shallow rounded lobes on the limb. In the pollen-mother cells, stained with acetocarmine, the dumbbell shaped chromosomes are constricted so that their number in some stages appears higher than the real one—$x = 12$. This was clear on permanent slides where, presumably due to the fixation, the chromosomes were shortened, less closely arranged, and their constriction invisible in the deep hematoxylin stain. The root tip cells contain 24 slender, straight or U-shaped chromosomes

N. solanifolia Wolf–*N. cardiophylla* Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised developed slowly to a short non-branched stem, with many long petioled, broadly ovate and highly undulate leaves. The corolla resembled that of *N. paniculata*, except for being larger and of cream yellow color. The pollen-mother cells stained with acetocarmine, show 12 chromosomes and the root tip sections on the permanent slides show 24 straight or U-shaped chromosomes. Under the name *N. solanifolia* were raised plants from seeds obtained from a commerical source which were quite different from those described above, and resembled more closely *N. rustica* var. *humilis*, except that the corolla was even smaller, somewhat different in shape and slightly violet-tinged on the tube. Their chromosome number was $x = 24$ or $2x = 48$.

The author gratefully acknowledges his indebtedness to Mr. SIDNEY YARNELL and DOCTOR GREGORY PINCUS for their assistance in preparing the manuscript for publication. Especially he wishes to thank Professor EDWARD M. EAST whose aid and helpful criticism made the work possible.

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DESCRIPTION OF PLATES

The drawings except figures 60, 65, 69, were made from single sections with the aid of a camera lucida. All figures in plates I and II were drawn by using the large Zeiss microscope with ocular 10 and oil immersion 1/16. Those of plates III and IV by using compensating ocular 12 instead of 10. As reproduced, all figures have a magnification of 1500 diameters.

PLATE 1

Figures 1–15 represent the polar view of the mitotic metaphase of the following *Nicotiana* species:

$2x=48$

FIGURE 1.—*N. Tabacum*, var. *macrophylla.*
FIGURE 2.—*N. rustica*, var. *brasilia.*
FIGURE 3.—*N. Bigelovii.*
FIGURE 4.—*N. nudicaulis.*

$2x=32$

FIGURE 5.—*N. suaveolens.*

$2x=24$

FIGURE 6.—*N. glutinosa.*
FIGURE 7.—*N. sylvestris.*
FIGURE 8.—*N. paniculata.*
FIGURE 9.—*N. glauca.*
FIGURE 10.—*N. tomentosa.*
FIGURE 11.—*N. trigonophylla.*
FIGURE 12.—*N. Palmeri* (?)

$2x=20$

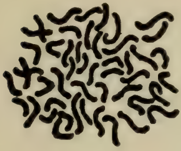
FIGURE 13.—*N. plumbaginifolia.*

$2x=16$

FIGURE 14.—*N. alata.*
FIGURE 15.—*N. Langsdorffii.*

CHRISTOFF, M., Cytological studies in Nicotiana

PLATE 1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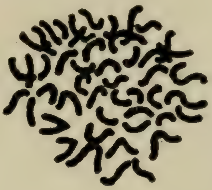
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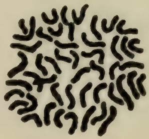
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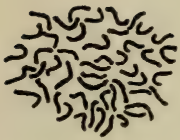
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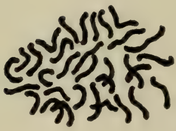
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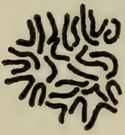
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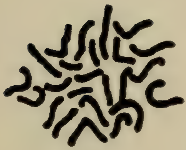
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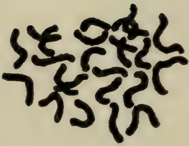
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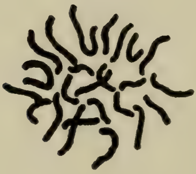
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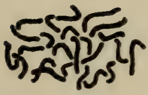
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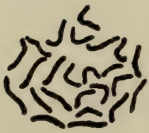
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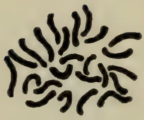
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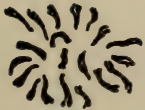
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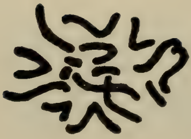
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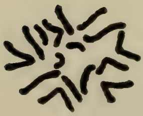
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Plate 2

Figures 16–33 represent the polar view of the heterotypic metaphase of the following Nicotiana species:

$x=24$ Chromosomes

Figure 76.—*N. Bigelovii.*
Figure 17.—*N. quadrivalvis.*
Figure 18.—*N. rustica*, var. *humilis.*
Figure 19—*N. Tabacum*, var. *macrophylla.*
Figure 20.—*N. viscosa* (?)
Figure 21.—*N. nudicaulis.*

$x=16$ chromosomes

Figure 22.—*N. suaveolens.*

$x=12$ chromosomes

Figure 23.—*N. glauca.*
Figure 24.—*N. sylvestris.*
Figure 25.—*N. paniculata.*
Figure 26.—*N. glutinosa.*
Figure 27.—*N. acuminata.*
Figure 28.—*N. trigonophylla.*
Figure 29.—*N. Palmeri* (?)

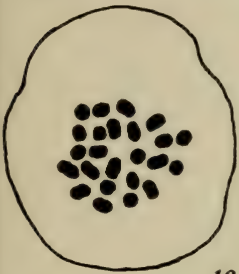
$x=10$ chromosomes.

Figure 30.—*N. longiflora.*

$x=8$ chromosomes.

Figure 31.—*N. Langsdorffii.*
Figure 32.—*N. alata.*
Figure 33.—*N. alata.*
Figure 34.—*N. alata*—homeotypic metaphase.
Figure 35.—*N. alata*—homeotypic metaphase.
Figure 36.—*N. alata*—heterotypic metaphase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 2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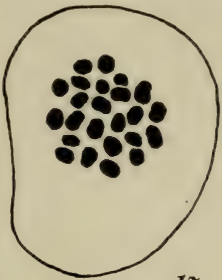
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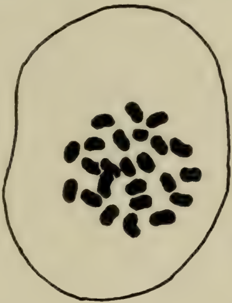
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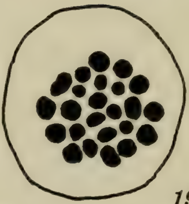
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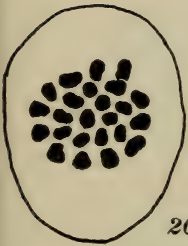
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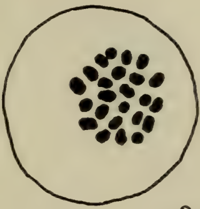
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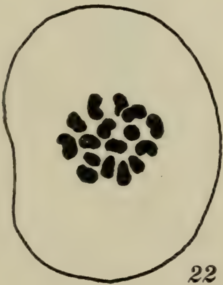
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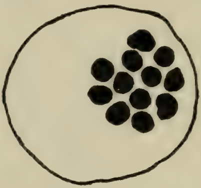
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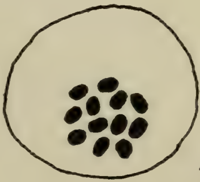
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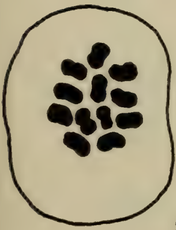
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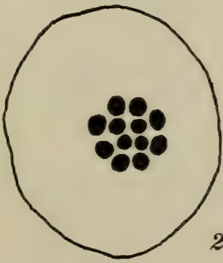
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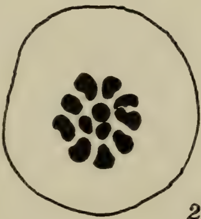
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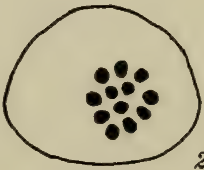
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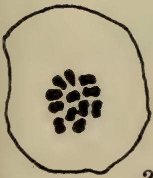
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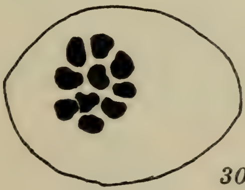
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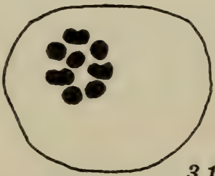
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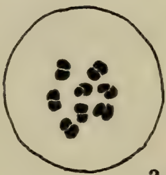
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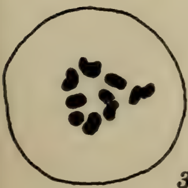
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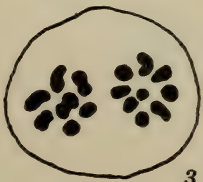
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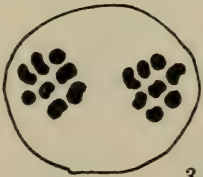
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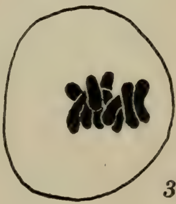
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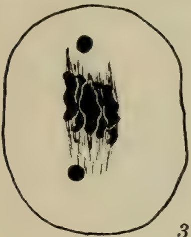
PLATE 3

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. longiflora ($x=10$)×*Sanderae* ($x=8$)
FIGURE 37.—heterotypic metaphase, side view.
FIGURE 38.—homeotypic metaphase.
FIGURE 39.—homeotypic metaphase.
FIGURE 40.—homeotypic anaphase.
N. Tabacum ($x=24$)×*alata* ($x=8$)
FIGURE 41.—very early heterotypic metaphase, polar view.
FIGURE 42.—very early heterotypic metaphase, side view.
FIGURE 43.—heterotypic metaphase, side view.
FIGURE 44.—late heterotypic metaphase.
FIGURE 45.—homeotypic metaphase, polar view.
FIGURE 46.—homeotypic anaphase, side view.
N. paniculata ($x=12$)×*Langsdorffii* ($x=8$)
FIGURE 47.—heterotypic spindle.
FIGURE 48.—heterotypic anaphase.
FIGURE 49.—homeotypic metaphase, polar view.
FIGURE 50.—homeotypic metaphase, polar view.
FIGURE 51.—homeotypic anaphase, side view.
N. rustica ($x=24$)×*paniculata* ($x=12$)
FIGURE 52.—heterotypic metaphase, polar view.
FIGURE 53.—heterotypic anaphase, side view.
FIGURE 54.—homeotypic metaphase, polar view.
FIGURE 55.—homeotypic metaphase, polar view.
FIGURE 56.—telophase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 3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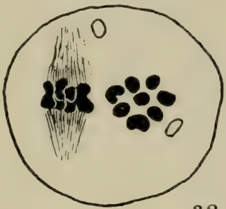
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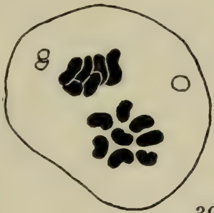
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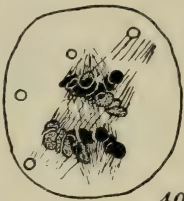
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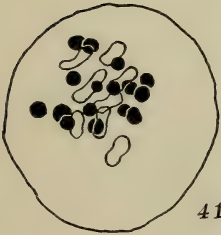
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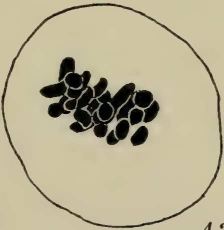
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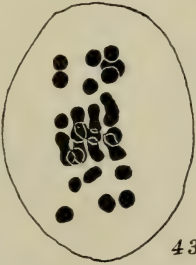
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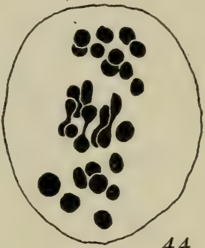
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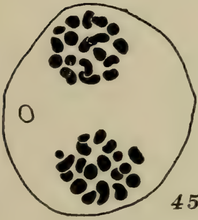
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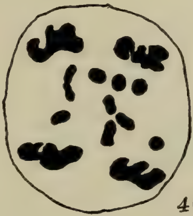
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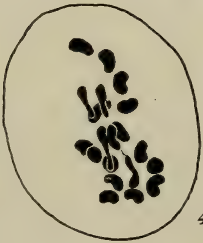
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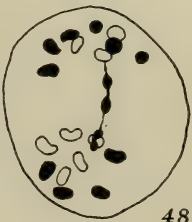
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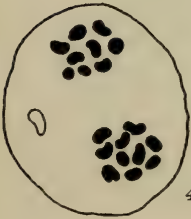
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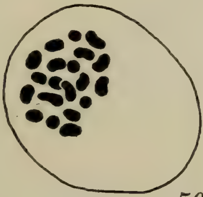
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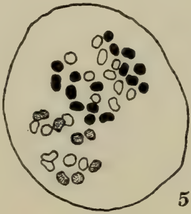
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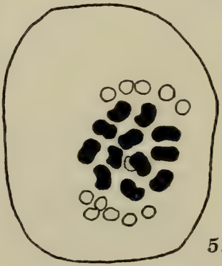
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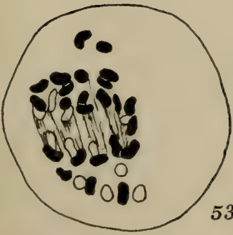
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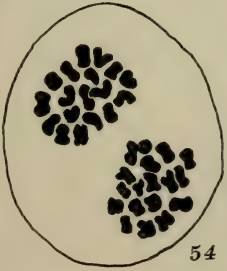
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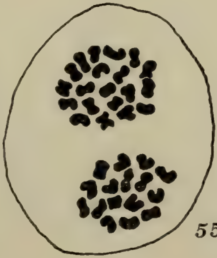
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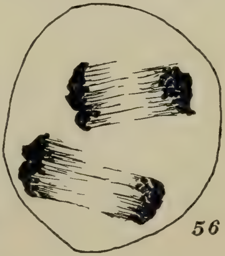
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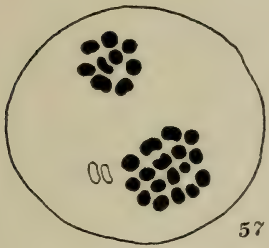
PLATE 4

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. suaveolens ($x=16$)×*glutinosa* ($x=12$)
FIGURE 57.—homeotypic metaphase, polar view.
FIGURE 58.—heterotypic metaphase,side view.
FIGURE 59.—homeotypic metaphase.
N. suaveolens ($x=16$)×*Bigelovii* ($x=24$)
FIGURES 60a & b.—heterotypic spindle.
FIGURE 61.—homeotypic metaphase, polar view.
FIGURE 62.—homeotypic metaphase, polar view.
FIGURE 63.—homeotypic spindles.
FIGURE 64.—octad.
N. rustica ($x=24$)×*Tabacum* var. *alba* ($x=24$)
FIGURE 65a & b.—heterotypic spindle.
FIGURE 66.—homeotypic anaphase.
FIGURE 67.—homeotypic metaphase, polar view.
FIGURE 68.—homeotypic metaphase, side view.
FIGURE 69, a, b, c.—homeotypic anaphase, polar view.

CHRISTOFF M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 4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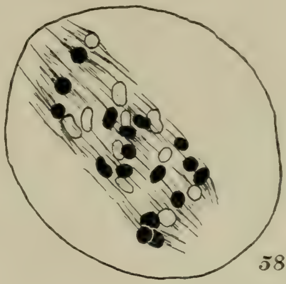
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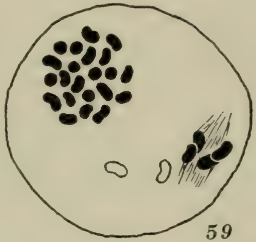
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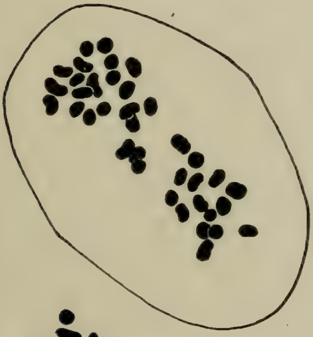
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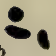
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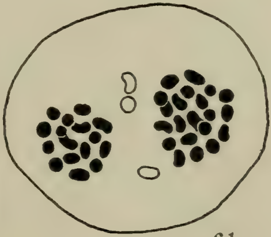
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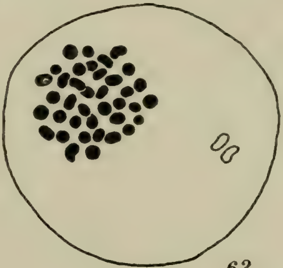
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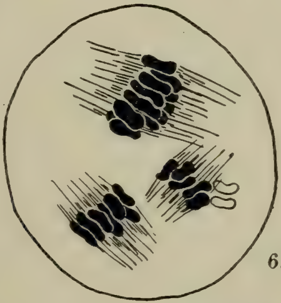
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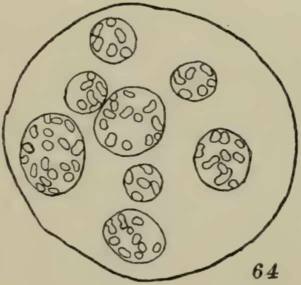
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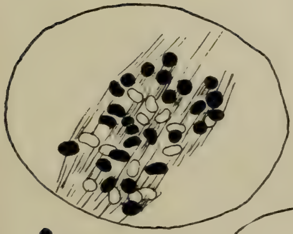
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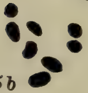
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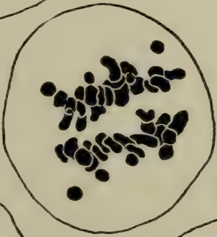
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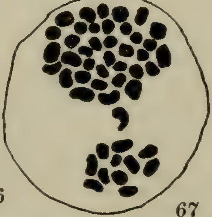
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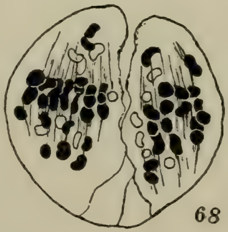
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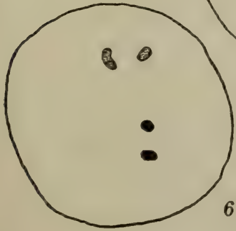
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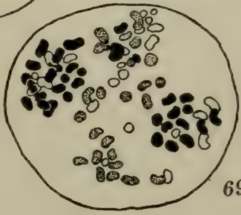
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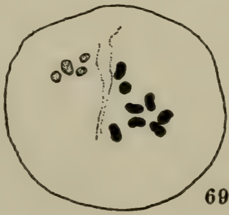
69c

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GENETICS, MAY, 1928

TABLE OF CONTENTS

PAGE

LIEN FANG CHAO, The disturbing effect of the glutinous gene in rice on a Mendelian ratio 191

BELAMY, A. W., Bionomic studies on certain teleosts (Poeciliinae). II. Color pattern inheritance and sex in *Platypoecilus maculatus* (Günth.) 226

CHRISTOFF, M., Cytological studies in the genus Nicotiana 233

[This number of GENETICS (**13: 191–277**) was issued June 1st, 1928]

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REPRINTED FROM
"PROTOPLASMA"
1928 Vol. IV No. 2
Verlag von Gebrüder Borntraeger in Leipzig

CONWAY ZIRKLE:

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INTERNATIONALE ZEITSCHRIFT FÜR PHYSIKALISCHE CHEMIE DES PROTOPLASTEN
INTERNATIONAL JOURNAL OF THE PHYSICAL CHEMISTRY OF PROTOPLASM
ARCHIVES INTERNATIONALES DE CHIMIE PHYSIQUE DU PROTOPLASMA
ARCHIVIO INTERNAZIONALE DI CHIMICA FISICA DEL PROTOPLASMA

UNTER BESONDERER MITWIRKUNG VON
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Alle biologischen Disziplinen mit kausaler Fragestellung sind an der physikalischen Chemie des Protoplasmas interessiert. Um das harmonische Zusammenarbeiten der verschiedenen Wissenszweige, um den Überblick und die Synthese auf dem Gebiete der Protoplasmaforschung zu ermöglichen, bedarf es eines eigenen die Ergebnisse zentralisierenden Organes.

PROTOPLASMA, die Internationale Zeitschrift für Physikalische Chemie des Protoplasten ist schon im ersten Jahre des Erscheinens zu diesem von Vielen längst ersehnten räumlich-geistigen Bande aller Protoplasma-Forscher geworden.

Die zunächst liegende Aufgabe der Internationalen Protoplasma-Zeitschrift wird es sein, die Schwierigkeiten des geistigen Kontaktes abzubauen, unter denen jeder einzelne Forscher und die gesamte Wissenschaft auf diesem Gebiete infolge der Heterogenität der beteiligten Disziplinen leiden. Die Physiologie der Pflanzen und Tiere, die allgemeine Cytologie, die verschiedenen medizinischen Wissenszweige (wie Physiologie, Pharmakologie, Pathologie, Histologie), die alle in der physikochemischen Erforschung des Protoplasmas die Lösung so mancher Rätsel erhoffen, haben den dringend gewordenen Austausch an Gedanken, Methoden, Erfahrungen bisher nur allzu spärlich und zögernd in die Wege geleitet.

Mit der Beseitigung der trennenden Schranken wird sich von selbst die Erreichung des wichtigsten Zieles der Internationalen Protoplasma-Zeitschrift ergeben: Durch die gegenseitige Anregung der einzelnen Disziplinen neue Blickpunkte zu gewinnen, neue Arbeitsmöglichkeiten zu schaffen.

Die Protoplasmaforschung muß geformt, geprägt, organisiert werden. Das kann heute nicht mehr ein Einzelner, sondern nur eine Arbeitsgemeinschaft, an der alle kausal-biologischen Disziplinen sowie die physikalische Chemie beteiligt sind.

Das Arbeitsgebiet der neuen Zeitschrift bedarf der Abgrenzung mit möglichster Schärfe. Die eigensten Gebiete der Protoplasmaforschung, die zu pflegen die neue Zeitschrift sich in erster Linie zur Aufgabe machen wird, seien besonders namhaft gemacht:

Kolloidchemie des Protoplasten. Physiko-chemische Eigenschaften des Protoplasten (wie Oberflächenspannung. Viskosität. Quellung. Elastizität. Adhäsion. Adsorption. pH. rH. Et cetera). Mikrochemie des Protoplasten im kausal-analytischen Sinne. Elektrometrie des Protoplasten. Vitale Protoplasmastruktur. Osmotische Zustandsgrößen des Protoplasten. Permeabilität. Plasmolyse. Narkose. Cytolyse. Hämolyse. Vitalfärbung. Physiko-chemische Grundlagen der Protoplasmabewegung. Microdissection. Ultra- und Polarisationsmikroskopie in Anwendung auf die Protoplasmaforschung. Mechanismus der Zell- und Kernteilung. Protoplasma-Aktivierung. Physiko-chemische Grundlagen der pharmakologischen und Gift-Wirkungen, der Resistenz und Empfindlichkeit sowie der Strahlenwirkung auf den Protoplasten. Pathologie des Protoplasten (Physikalische Chemie des Tumorprotoplasten). Modellversuche an leblosen Kolloiden, insofern sie zur Klärung der Protoplasteneigenschaften beitragen.

Die Zeitschrift erscheint in zwanglosen Heften, von denen 4—5 einen Band von 40 Druckbogen bilden. Die Abhandlungen, Kleineren Mitteilungen, Sammelreferate und kritischen Referate können in deutscher, englischer, französischer oder italienischer Sprache verfaßt sein.

Subskribenten werden die einzelnen Hefte zu einem Vorzugspreis geliefert, der nach Erscheinen des Schlußheftes eines Bandes erlischt. Der Preis des ganzen Bandes erfährt somit für Nicht-Subskribenten eine Erhöhung.

THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THE FIXATION IMAGE OF VARIOUS SALTS OF CHROMIUM

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With Plates V—VIII

Received for publication, March 7, 1928

It was early observed that the destruction of certain cell organs by the process of dehydration, embedding and cutting was greatly lessened by a preliminary treatment with certain compounds of chromium. The microscopic structure of the cell organs thus preserved, however, was seldom that which they had in living tissue. The addition of other reagents, acetic acid, osmium tetroxide, formaldehyde, etc., to the chromium solutions corrected this fault to some extent, with the result that the more successful fixing fluids are compounds of more or less complexity. Chrome fixation is thus not a simple process, and there is little agreement among cytologists as to what chemical or physical reactions are involved.

The particular cell elements which are preserved by the chromium derivatives depend to a certain extent upon the compound used. Thus chromic acid is generally used in mixtures designed to fix nuclei, and potassium bichromate in those which fix cytoplasm. FLEMMING (8) was quite emphatic about the use of bichromates in studies of the nucleus during cell division, stating, „Wer mit Kalibichromicum oder anderen Chromsalzen Kernteilungen suchen, oder ausschließen will, begibt sich auf einen hoffnungslosen Irrweg.“ BURCHARDT (2), on the contrary, reported that only certain bichromates destroyed nuclei. The bichromates of sodium, ammonium, magnesium, strontium and zinc were as destructive as potassium bichromate, while those of barium, calcium and copper preserved the nuclei as well as the cytoplasm. Acetic acid, he held, should always be added not only to insure the correct fixation of the nucleus but also to enhance penetration and the preservation of cytoplasm. A mixture of potassium bichromate, chromic acid and acetic acid was recommended as a good fixative for dividing nuclei. For the fixation of cytoplasm, ALTMANN (1) used a mixture of osmium tetroxide and potassium bichromate and stated that the bichromate should contain no free chromic acid. Thus the work of some of the earlier cytologists indicated that those chromium compounds which best preserved the cytoplasm were more alkaline than those which gave the best results with nuclei. In keeping with this is the work of YAMAHA (22) who reported that the pH of the common nuclear fixatives ranged from 1·1 to 3·0 and that, with the exception of BENDA's (pH 1.2) and CHAMPY's (pH 1·4) the mitochondria fixatives had a pH greater than 3·0.

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Recently several important investigations have been made of the effect of pH upon the solubility of various cell elements both with and without previous fixation, and upon the ability of the elements when fixed to take up and retain certain stains. NAYLOR (18) fixed a number of root tips with chrom-acetic acid and found that the tissue retained acid dyes in acid solutions (pH 3·4—4·6) but lost them in more alkaline solutions (pH 5·0—7·3). Basic dyes were retained in tissues buffered from pH 5·6—7·3 but were lost in more acid solutions. The isoelectric points of the various cell proteins showed quite a range. At pH 5·1 the nucleolus and chromosomes united with the cation, while the resting chromatin united with the anion. The isoelectric point of the cytoplasm, at least of those cytoplasmic elements which survived the chrom-acetic acid fixation, was much more acid (pH 4·3) than that of the chromosomes and nucleoli (pH 5·6). EARL (6) fixed root tips of *Vicia faba* with CARNOY's absolute alcohol-chloroform-acetic acid solution and thus avoided any possible combination of the cell proteins with any of the heavy elements present in the more usual fixing fluids. Treating sections with NaH_2PO_4 brought to pH 5·0 by titrating with NaOH caused no appreciable effect, though when the sections were soaked in 1% Na_3PO_4 or NaOH of the same pH the chromosomes were caused to swell and loose their staining capacity. The chromatin was apparently dissolved. KUWADA and SAKAMURA (11) found that in unfixed material the chromosomes were dissolved by a mixture of oxalic acid and potassium oxalate when its alkalinity was greater than pH 5·6. Likewise the mitochondria were influenced by the hydrogen-ion pressure (COWDRY 3). LEWIS and LEWIS (13) found in their tissue cultures that an acid medium (pH 4·4) caused the mitochondria to become varicose and even to swell into vesicles. When the tissue was bathed in an alkaline solution (pH 9·6) they shortened into thick rods and granules.

In the present investigation a 2·5 % solution of chromium trioxide was taken as the basis of all the bichromate fixing fluids. The cation was added as a carbonate, oxide or hydroxide. Those elements whose chromates are insoluble or very sparingly soluble were always added in excess and thus each bichromate solution buffered at its characteristic pH. Elements whose chromates were soluble, potassium, sodium, &c., were, of course, not so buffered, but were used at the pH of their bichromates. In brief, all of the fixation images could be classified into two groups depending upon the pH of the fixative (ZIRKLE 24). Certain bichromates such as barium, mercury, &c., were so acid that their fixation image was in general that of chromic acid. Others, such as potassium, stronctium, &c., gave the tissue two distict images. If their solutions were on the acid side of a certain point, specific for each cation, the image was practically that of chromic acid; if the solutions were on the alkaline side of this point, the fixation image was quite different and characteristic of the positive element. These two images will here be referred to as "acid" and "basic", though it should be noted that the "basic" fixation image is given by solutions which, without exception, are more acid than pH 7·0.

The exact point of change from an acid to basic fixation image was not determined in every case because of the seemingly erratic character of the fixation near that point. Different specimens fixed in the same fluid, dehydrated and embedded together, would have the one an acid, the other a basic fixation image. The same fluid would give on one day an acid fixation image, on another a basic one. Once the critical point was passed, however, a relatively large change in pH would have no appreciable effect.

The pH values recorded for the several bichromate solutions are only approximately correct. They were determined colorimetrically and as the chromate ion was itself colored, exact matching of shades was not feasible. As there were nearly always two and sometimes three different indicators used, this error is perhaps not great. Another source of error was the possible occurrence of a chemical combination between the acid salt and the indicators. The electrometric method was not used because of the unknown influence of the ions of the heavier metals. The determinations were made with sufficient accuracy, however, to establish (1) that there is a critical point in the pH of a bichromate solution where the fixation image changes from an acid to a basic one and (2) that this point varies greatly in the different bichromates.

The various forms assumed by certain cytoplasmic organs in the different fixatives makes it expedient to redefine provisionally certain terms. Mitochondria will here include not only those rod or thread like structures which occur in cytoplasm fixed by potassium bichromate, but also any granules, chains of granules and spheroids which appear where certain fixatives are used, when it can be shown that these latter forms are assumed by the structures which usually appear rod or thread like. The term plastid will be applied only to those cytoplasmic organs which contain starch grains, or which can be shown to be able to store starch.

Material was left in the fixative from 36—40 hours, and was stained in HAIDENHAIN's iron-alum haematoxytin. Root tips of *Zea maize* were used throughout.

CHROMIC ACID

Considering the fact that chromic acid is perhaps more widely used as a constituent of fixing fluids than any other reagent, it would seem that any description of its fixation image would be redundant. It is described here for two reasons; first, because it has generally been used in combination with other reagents, acetic acid, formalin, etc., and when so combined does not give its characteristic fixation image, and second, its fixation image is typical of all of the very acid salts of chromium. Compared with the reagents with which it is usually mixed, chromic acid penetrates relatively slowly, and evidence will later be given which indicates that the fixation image is almost entirely determined by the first lethal substance to reach the tissue. Thus in chrom-acetic fixatives the chromic acid serves primarily for a post-chromatization of the acetic acid fixation image.

The most striking artifact in tissue fixed with chromic acid (2·5 % pH $\langle$ 1·0) is the shrinkage of the protoplast (Fig. 4). The cell walls are not mordanted for HAIDENHAIN's haematoxylin and hence the tissue appears to be almost completely macerated. This shrinkage is not due to the chromic acid itself but to the subsequent dehydration and embedding, for when tissue thus fixed has been soaked for 36 hours in $CuCr_2O_7$ (pH 4·6), practically no shrinkage is evident. The cell walls are also mordanted and the tissue does not appear macerated. Chromic acid mordants starch grains so that they stain a dark blue with haematoxylin, though the enclosing plastids are dissolved. In the root cap they are quite conspicuous (Fig. 1). This mordanting is dependant upon the fixative being quite acid.

Cytoplasm is disorganized by chromic acid, only certain stringy elements (spongioplasm?) being preserved. The whole has an extremely washed out appearance. Mitochondria are nowhere preserved, though they are destroyed probably only in the outer part of the tissue, for when the fixed material is treated subsequently with $CuCr_2O_7$ (pH 4·6) some mitochondria are found in the central cylinder. Their form and number are hard to distinguish as they are entangled with and obscured by the disorganized cytoplasm. In resting cells the nucleolus is fixed as a darkly staining sphere and is located near the center of a hollow nucleus, whose surface is composed of a chromatin reticulum (Fig. 4). This reticulum is coarse and varies greatly from cell to cell, so precisely what it represents in the living tissue is uncertain. The nuclear lymph is completely dissolved. In dividing cells the chromosomes tend to be clumped, though the spindle fibres are individually recognizable. In prophase, the spireme is distinguishable.

POTASSIUM BICHROMATE

Like chromic acid, potassium bichromate in the more useful fixing fluids is generally mixed with reagents which penetrate the tissue more rapidly. Thus tissue fixed in REGAUD's (bichromate and formalin) is probably fixed first by the formalin, though proof of this is at present lacking, for the fixation image of formalin post-chromatized with potassium bichromate so closely resembles the fixation image of the bichromate that they cannot with certainty be separated. The main difference between REGAUD's and potassium bichromate is that the former fixes the whole tissue while the latter can be relied upon to fix only the cells near the periphery. Of course what reactions actually occur durin fixation with such a solution as REGAUD's composed of strong oxidizing and strong reducing substances, can not at present be recognized. Mixed with osmium tetroxide (ALTMANN's fixative) potassium bichromate seems to prevent the osmium from being reduced except by the true fats and mixtures of cholesterin and unstaurated fatty acids. It would seem that in this fluid the constituents penetrate at nearly the same speed. ERLIKI's fixative (bichromate and copper sulphate) gives practically the same fixation image as the bichromate alone (ZIRKLE 23).

When mixed with chromic acid, potassium bichromate gives the typical acid fixation image. If the mixture is on the alkaline side of pH 2·0 the shrinkage so obvious with chromic acid fixation will be greatly lessened. Otherwise the image is that of chromic acid. When tissue is fixed in a 2·5% solution of bichromate containing no free acid pH 4·4) the image is typically basic (Figs. 5, 25). The nucleolus is a darkly staining spheroid just as it is after the acid fixation. The nuclear lymph is well fixed, does not stain as darkly as does the nucleolus, so the resting nucleus appears as a solid lightly staining body with a very dark nucleolus centrally located. There is here no trace of the hollow resting nucleus of acid fixation. The mitochondria are well fixed in the outer part of the tissue, though due to the slowness of the bichromate's penetration, they are rarely fixed in the central cylinder. They are fixed as short, stout threads or rods. (Figs. 5, 25). Occasionally they fix as small spheres, though whether this is due to an erractic behavior of the fixative or to an actual change of their shape in the living tissue is not known. Back from the growing point the mitochondria can be observed developing into plastids (Fig. 25). It is often possible to get mitochondria, transitional stages and plastids in the same microscopic field. The alteration caused by the potassium bichromate in the structure of certain mature plastids has been recorded (Zirkle 23). In the central cylinder, about 5 c. m. back from the root tip, two distinct types of mitochondria can be observed, the larger developing into plastids and the smaller apparently undergoing no further changes (Fig. 6). This is in complete harmony with the findings of Guilliermond (9).

All chromatin is destroyed by the fixative. The evidence would indicate that the nuclear lymph permeates the chromatin, for in resting cells no vacuoles appear in the nuclear regions usually filled by the chromatin reticulums. As the spindle fibers are also destroyed, there can be no fixation of dividing nuclei. Indeed, there is evidence that if nuclei are dividing when the bichromate penetrates the cell, they are actually made to appear to be resting. If the chromosomes and spindle fibers are dissolved, the nuclear lymph fixed as a spherical mass and the nucleolar material clumped into from one to three globules, the fixation image will be same as that of a resting nucleus.

The cytoplasm is comparatively well fixed. The hyaloplasm is fixed, though whether the spongioplasm is also fixed is uncertain. The cytoplasm stained appears finely granular with sharply delineated vacuoles. The cellulose cellwalls are mordanted so there is no appearance of maceration. Starch grains are not mordanted, and having nearly the same index of refraction as the balsam in which they are mounted, they are nearly invisible.

While a considerable change in the pH of the fixatives has little influence on the fixation image once the critical point is passed, a noticeable change will occur if the alkalinity is increased up to pH 6·0. The mitochondria here tend to become small spheres and the whole nucleus stains a dense black.

COPPER-, GLUCINUM- AND CERIUM-BICHROMATE

When the pH of a mixture of chromic acid and potassium chromate is raised to about 4·4, there is a sudden change in the type of fixation image that it gives. The acid image of chromic acid becomes the basic image of potassium bichromate. The exact pH at this change is uncertain because of certain uncontrolled factors. There is, however, little or no overlapping of fixation types, the images being either acid or basic. When certain other elements are substituted for potassium, some of the characteristics of the basic fixation image appear before all of the acid fixated structures have disappeared, and the resulting overlapping of types gives an image which seems much more complete than those of the usual fixatives.

A 2·5% solution of copper bichromate (EIMER and AMEND), pH about 2·0, gives the fixation image of chromic acid. When an excess of cupric oxide is added, the pH is changed to 4·6 and the resulting image combines elements of both acid and basic fixation. In resting cells the nucleolus is fixed as a darkly staining sphere in the center of a solid nucleus of fixed nuclear lymph. Only rarely is the resting chromatin preserved. The reliability of this fixation for chromosomes increases as the cell division progresses. The various spireme stages show clearly as a rule, though at times it is not until the chromosomes are formed that the chromatin fixation is entirely reliable. Chromosomes at metaphase are always well fixed (Fig. 2). While the spindle fibers are not distinguishable individually, collectively they are well delineated. The mitochondria are well fixed and as the various structures of the dividing nuclei are also preserved, it is possible to trace the mitochondria through the various stages of cell division. The cytoplasm is as well fixed as when potassium bichromate is used.

The whole tissue, however, is not equally well preserved. The copper bichromate penetrates well and the fixation of the central cylinder is generally satisfactory. The outer cells and particularly the epidermis are frequently disorganized. This disorganization is probably not due to destructive action of the fixing fluid, for if it is washed out with 70% alcohol and dehydration proceeds rapidly, these outer cells will generally appear to be nearly as well fixed as those further in the interior. The addition of 1% copper sulphate makes the fixative equally good for all of the tissue. It can then be washed out in water and dehydration can proceed normally. (See appendix).

If to a 2·5% solution of copper bichromate a like amount of a 2·5% solution of copper acetate be added, the mixture will buffer in the presence of a slight excess of copper oxide at about pH 4·6. While the hydrogen-ion pressure of the mixture is the same as that of the copper bichromate, its fixation image is entirely acid. There is in tissue thus fixed no trace of mitochondria or nuclear lymph. The resting chromatin is well preserved, the spindle fibers distinct and the chromosomes and spireme especially clear. The fixation image is very close to that of chrom-acetic acid. It is only in the fixation of the nucleolus that copper seems to influence the fixation. Nucleoli thus fixed cannot be cut in paraffin section. When struck by the microtome knife they are knocked out of the nucleus and at times even out of the cell (Figs. 3, 7). They can frequently be seen connected to the spireme (Fig. 8) and seem to flow into it as the cell division progresses. The presence of as much as ·4% copper acetate in the fixative will dissolve all mitochondria and even as little as ·2% will cause the resting chromatin to be fixed.

While copper bichromate will not fix mitochondria and nuclear lymph in solutions more acid than pH 4.6, once the fixation has occured, an increased acidity of the solution will not appreciably alter the image. Root tips fixed 36 hours in copper bichromate, washed and soaked for 36 hours in chromic acid had the fixation image of the bichromate. When the procedure was reversed, the image was essentially that of the chromic acid except, as has been stated, that the usual shrinkage of the protoplast was absent and there were traces of mitochondria in the disorganized cytoplasm of the central cylinder. It should be emphasized that the fixation image is determined not only by the substances in the fixative but also by the order in which the various constituents reach the tissue.

Certain resemblances of chrome fixation to chrome tanning will be discussed later. An important difference between the two processes will be pointed out here. Chrome tanned leather can be untanned by soaking in a solution of Rochelle Salts (Wilson 2). Root tips fixed 36 hours in copper bichromate were washed and placed in a 2% solution of Rochelle Salt ($NaKC_2O_4$) over night. All of the colored chromium ions were apparently removed from the tissue and it appeared quite white until cleared in xylene, when its faint green tinge showed the presence of a trace of the chromic ion. The material was not unfixed by the treatment and only differed from the control by having a trifle more granular cytoplasm.

When sections of root tips fixed in copper bichromate are mordanted in a 2·5% solution of the fixative instead of the usual 2% Iron Alum (pH 3·0) before being stained in Haematoxylen, the tissue is stained a dark brown instead of the familiar blue. When destained in Iron Alum the sections turn the usual dark blue. The chromatin is stained as usual but the nucleolus, instead of being the darkest object in the cell, is almost completely unstained. It is a very light brown in contrast to the dark blue chromatin. The spireme in tissue thus treated often appears as a tube with an unstained center, suggesting that when the nucleus disappears as a distinct cell organ it flows into the spireme.

If a slight excess of glucinum carbonate be added to a 2·5% solution of chromic acid, the resulting glucinum bichromate buffers at about pH 4·6. This solution gives practically the same fixation image as copper bichromate (Fig. 12). The mitochondria thus fixed are slightly stouter and shorter than when fixed with copper. There is a slight tendency for the resting chromatin to be fixed and the nuclear lymph is not so regularly fixed as with copper. Otherwise the two fixation images are alike. There is a difference in the penetrating power of the two fixatives, the glucinum bichromate penetrating very badly. As a result, the cells best fixed are near the periphery and outer cortex. The glucinum fixation of the central cylinder is unreliable.

Cerium bichromate (pH 4·8) formed by the addition of a slight excess of cerium carbonate to a 2·5% solution of chromic acid, gives a fixation image a trifle more basic than does copper or glucinum. Mitochondria are fixed as in glucinum bichromate and dividing nuclei are just as well preserved as when fixed in glucinum or copper. The nuclear lymph is regularly well fixed and the resting chromatin never preserved. Like glucinum bichromate, it does not penetrate the tissue readily so the best fixation occurs near the periphery (Fig. 11).

LITHIUM BICHROMATE

A 2·5% solution of lithium bichromate C. P. (EIMER and AMEND) has a pH of 4·6. It fixes the epidermal layer very much as does potassium bichromate: i. e. mitochondria, nuclear lymph, nucleoli well fixed, all chromatin dissolved. In the interior, however, few mitochondria are fixed and the fixation of nuclear lymph and nucleoli becomes quite erratic. The alkalinity of the solution can be increased to about 7·4 by the addition of lithium carbonate. Throughout the range from pH 4·8—7·4 the fixation image shows little variation and has only a very slight resemblance to the basic images of the other bichromates. The lithium fixation image is at present unique.

Perhaps the most striking characteristic of root tips fixed with lithium bichromate is the swollen nuclei, whose diameters are at least half again as large as in the living cells (Fig. 9). The nucleoli, instead of being spheroids, as they usually are when fixed at this range of hydrogen ion concentration, are amoeboid with rather pointed pseudopodia, (Figs. 9, 10) from whose tips branching strands run out to the nuclear membrane. Except in the epidermis, whose fixation is characteristically basic, no nuclear lymph is fixed. As all the chromatin is also dissolved, the nuclei appear as large spherical masses, hollow except for the nucleolus and the strands connecting it to the nuclear surface. Spindle fibers are dissolved and nowhere is there any indication of cell division. The mitochondria in the root tip are spherical (Fig. 9) and greatly resemble minute plastids. This tendency of the mitochondria to contract into spheres as a result of an increased alkalinity of the fixing fluid, is evident in other bichromates and certainly here it is not due entirely to the lithium. Their form seems much more stable back in the region of differentiation and here they are sometimes fixed as rods. It is thus possible to trace "plastids" developing back into mitochondria as a collollary of this particular kind of fixation. The cytoplasm has as essentially basic fixation, although it is somewhat disorganized.

No ready explanation for this peculiar reaction of lithium bichromate is available. It seems obvious that other cations beside hydrogen influence the double decomposition and recombination of salts and amphoteric proteins. Thus far lithium forms the only bichromate which does not fix the nuclear lymph when in solution more alkaline than pH 4·6. We must assume that unlike the compounds of sodium and potassium, any compound which may be formed by lithium and nuclear lymph is soluble in the fixative, water or the dehydrating alcohols. The presence of some lithium carbonate in the fixing fluid does not account for this fixation image. If the pH of lithium bichromate is raised above 4·6 by the addition of lithium in another form, the fixation image is the same.

BICHROMATES OF BARIUM, BISMUTH, ALUMINUM, LEAD, IRON AND MERCURY

The above elements as cations will form only very acid compounds with the bichromate ion and consequently the salts thus formed can give no basic fixation image. Indeed certain of these elements, even when present in excess, influence the fixing fluid so slightly that tissue fixed in their bichromates cannot be distinguished from that fixed in chromic acid. The pH of these solutions has not been accurately determined. In every case it was found to be less than 2·0.

When roots tips were fixed in the bichromates resulting from an addition of a slight excess of barium hydroxide, aluminum hydroxide, bismuth subcarbonate or lead carbonate to a 2·5 % solution of chromium trioxide, the resulting images were identical with that of chromic acid. Especially striking was the characteristic shrinkage of the protoplast and the mordanting of the starch grains in the root cap so that they would be stained with HAIDENHAIN's haematoxlyn. Ferric bichromate (slight excess of $Fe(OH)_3$ or Fe_2O_3 to a 2·5 % solution of CrO) had an acid fixation image which was, however, distinct from that of chromic acid. The shrinkage of the protoplast was greatly lessened, although still great enough to make the tissue appear somewhat macerated (Fig. 13). Only the centers of the starch grains in the root-cap were mordanted, their peripheral layer being unstained by haematoxylin. There was no trace of nuclear lymph, hyaloplasm or any structure which could be identified as mitochondria, although there were numerous small granules in the cytoplasm which are not found in tissue fixed with the usual acid fixatives.

Material fixed in mercuric bichromate (2·5 % sol. CrO_3 plus a slight excess of HgO) is very brittle and hard to handle. When dehydrated, it is likely to disintegrate at the slightest touch. It is stained a dark brown by the fixative and does not become transparent in xylene. There seems to be an affinity between the mercury and the cell walls. When the plumule of zea is fixed with the other bichromates, the younger leaves immediately surrounding the stem growing point shrink slightly during dehydration; the outer leaves do not. Thus, although the sheath of leaves was always cut straight across just above the tip of the stem, the cut ends of the leaves formed a concave surface before the dehydration was completed. When the fixing fluid was washed out with a 70% or stronger solution of alcohol this shrinkage was much less than when it was washed out with water. Mercury bichromate, however, so fixes this tissue that there is no shrinkage of the younger leaves and even when it is washed out with water the cut ends of the leaves remain in one plane.

The fixation image of mercury bichromate is much like those of the other elements described in this section. There is the usual shrinking of the protoplast and slight mordanting of the starch grains and the usual acid fixation image. In two respects, however, the mercury image is unique. First, it stains the lignified cell walls of the vascular bundles, especially the spiral thickening of the larger vessels. Second, it so fixes the nucleoli that they cannot be stained with HAIDENHAIN's haemotoxylin although the chromatin is stained black, (Fig. 14) thus furnishing additional evidence of the different properties of the nucleoli and the chromatin.

BICHROMATES OF SODIUM, AMMONIUM, STRONTIUM, CALCIUM, MAGNESIUM, CADMIUM AND ZINC

Sodium bichromate (pH 4·4) reacts throughout like potassium. Mixed with free chromic acid it gives an acid fixation image, at pH 4·4 its fixation image is basic. As its alkalinity is increased, the mitochondria are fixed as shorter and thicker rods until at neutrality they are small prolate spheres. Neutral sodium chromate also mordants the nucleus in such a way that it is stained a dense black with HAIDENHAIN's haematoxylin, and as a result its finer structures can be distinguished.

Ammonium bichromate fixes much as do sodium and potassium. The change from acid to basic fixation occurs at pH 4·2, the most acid point of change of any of the bichromates so far investigated. The acidity of this point, however, may have no significance, for ammonium penetrates the tissue so rapidly that there is no evidence for assuming that the interior of the root tip, where the fixation is best, is fixed at the pH of the exterior fluid. This relatively rapid penetration of ammonium as contrasted with potassium is perhaps responsible for the different regions the two salts fix. Potassium bichromate is a reliable fixative only for the peripheral layers of the root tip, while ammonium bichromate fixes only the interior satisfactorily. They thus supplement each other and a mixture of the two will fix the entire tissue. Cells fixed with ammonium bichromate appear very like those fixed with sodium and potassium, the only marked differenc being in the mitochondria which are much more slender and threadlike when fixed with ammonium (Figs. 15, 17).

When the pH of a solution of lead bichromate is raised to 4·4 by the addition of ammonium hydroxide, its fixing properties are apparently determined primarily by the ammonium bichromate formed, for its fixation image is almost exactly like that of pure ammonium bichromate, the one notable exception being the preservation of greatly swollen chromosomes at metaphase. All other chromatin is dissolved as it is in ammonium bichromate.

When a 3 % solution of silver nitrate is mixed with an equal volume of 4 % ammonium bichromate, there is thrown down a dark red precipitate of silver chromate. The clear orange colored filtrate (pH 3·0) contains among other compounds the complex ammonia-argentic ion ($Ag(NH_3)_2^+$). Root tips fixed in this fluid have the regulation acid fixation image except that mitochondria are preserved in the central cylinder in the form of slender rods and threads. They seem to be well preserved but are difficult to distinguish clearly because of the extreme disorganization of the enclosing spongioplasm. When the tissue is dehydrated in the light, metallic silver is reduced and occurs as minute crystals in the cytoplasm and along the cell walls. When the pH of the filtrate is brought up to 4·4 by the addition of ammonium hydroxide, it fixes the peripheral three layers of cells in the root tip with an acid fixation image, and the inner cells with a basic one (Fig. 19). The fact that two images in inner and outer regions of the tissue result from a single fixation, can best be explained by assuming a difference in the rate of penetration of the toxic constituents of the fixing fluid. Postulating such a differential in the rate of penetration, it would follow that the constituents would penetrate the outer layers relatively close together, but would reach the inner ones some time apart. The first to fix the tissue would determine the fixation image. Ammonium bichromate gives a basic fixation image at the pH here used, and as ammonium compounds are known to penetrate with exceptional rapidity, the occurance of a basic fixation image in the interior of the root tip is in harmony with our postulate.

Strontium bichromate (pH 5·8), calcium bichromate (pH 5·6) and magnesium bichromate (pH 6·2) fix the root tips so nearly alike that it is not possible to determine which of them was used by an examination of the tissue fixed. The fixation images are typically basic yet quite distinct from the basic fixation images of potassium-, sodium- and ammonium-bichromates. The chromatin and spindle fibers are completely dissolved so there are no traces of dividing cells. The nuclear lymph, nucleoli and mitochondria are well fixed. The nucleoli are fixed as densely staining spheres which are much smaller than when they are fixed with bichromates of the potassium group (Figs. 16, 18). The mitochondria are also fixed differently for with these elements of the second group of the periodic system, they are small granules in the root tips (Fig. 18), and granules and streptococcus-like chains of granules in the region of elongation (Fig. 16). The bichromate of strontium apparently does not penetrate as well as those of calcium and magnesium, for its fixation of the inner cells of the root tip is somewhat erratic. Cadmium bichromate (pH 5·6) belongs with this group, yet differs in that it preserves traces of the chromosomes (Fig. 20) and thus gives a fixation image between those here described and that of zinc bichromate.

Zinc bichromate (pH 5·2) fixes resting nuclei and mitochondria as do the alkali-earth elements. In addition, it fixes the chromosomes at metaphase. Thus fixed, they are not the usual rod-like structure, but are vesicles, hollow prolate spheroids (Fig. 21). This pH (5·2) is the most basic one found in the present investigation at which chromatin can be fixed. It should be emphasized here that certain of the differences occuring in tissue fixed with various bichromates may not be due directly to the elements furnishing the cations, but to the fact that the several bichromates buffer at different hydrogen-ion concentrations. Only those differences in fixation image which persist when the bichromates, being compared, are at the same pH, can be considered as due to the specific cation.

CHROMIUM USED AS A CATION

In the fixation thus far described, chromium has participated as either a chromate or bichromate. It has been reported that the chromic ion unites with the skin proteins in chrome tanning (Wilson 21), so it seemed probable that a chromic salt would fix root tips. A 5 % solution of chromic sulphate $Cr_2(SO_4)_2 \cdot 15\ H_2O$ is quite acid, having a pH of about 2·0. What fixing it does is, of course, acid, though the image is so "washed out" that little except chromatin can be recognized. If, however, a slight excess of cupric oxide is added, the resulting mixture has a pH of 4·6 and give an excellent acid fixation image. Every cell organ so far mentioned except the nuclear lymph and mitochondria is preserved (Fig. 23). The various prophases are clearly fixed as is the hyaloplasm which here appears to be faintly granular. A solution of copper sulphate at pH 4·6 will not fix any of the organs mentioned and indeed will so disorganize the cell that practically none of its contents can be recognized. The addition of 4 % formaldehyde to the mixture, while it does not alter the pH, causes it to fix in addition both the nuclear lymph and mitochondria in the central cylinder (Fig. 22). It is for this region a very good universal fixative as the vacuoles in the cytoplasm are clearly delineated so that their growth and development from the growing point can be clearly followed. As formaldehyde penetrates so rapidly, it may well be that this latter fixation image is merely that of the formalin post-chromatized. If the formalin is given an hour head start, the entire root tip will be fixed as the mixture fixes the central cylinder.

A fixing fluid composed of copper bichromate, copper oxide and chromic sulphate at pH 4·6, containing as it does chromium both as anion and cation, is a very satisfactory one for general use.

A mixture of 5 % acetic acid and 20 % formalin gives an acid fixation image (MASCRE 15). If, however, the tissue is placed in the formaldehyde an hour before it is put in the mixture, the nuclear lymph and mitochondria will also be fixed. Thus it seems that in fixation the first toxic substance to reach the tissue is apt to determine the fixation image.

DISCUSSION

The findings of the present investigation make it evident that chrome fixation is not a simple process but one which involves several reactions which, as far as is known, may occur simultaneously or successively. It certainly does not consist in a mere precipitation of the liquid and semi-liquid proteins of the cell, as FISCHER (7) held. There seems to be an actual combination between certain elements of the fixing fluid and tissue fixed, as is indicated by the fact that tests for the presence of these elements give positive results after the tissue has been thoroughly washed. Whether or not a particular combination occurs depends upon the pH of the fixing fluid and, in the light of our present knowledge of the effect of pH upon the combination of protein with salts (Loeb 14), it is hardly probable that these elements are merely held in the tissue mechanically.

If the various cell organs were composed in whole or in part of amphoteric proteins, each with its characteristic isoelectric point, we might expect that, when the fixing fluid is on the acid side of these points, combination would occur between the proteins and the cations.

The nucleolus is fixed by both acid and basic fixatives. It regularly appears as a very darkly staining spherical body, and at times becemes so hard that it cannot be cut by the microtome knife but, when struck by the knife, is knocked out of the cell (Fig. 7). When fixed with $HgCr_2O_7$ it loses its staining capacity with Haidenhain's haematoxylin and appears only a light muddy brown, while the chromatin stains black. It is also stained very lightly when fixed with silver ammonium bichromate of pH 3·0. When fixed with copper bichromate (pH 4·6) and mordanted with iron alum, it is the most heavily staining organ in the cell, but when so fixed and mordanted with the fixative, it is not stained with haematoxylin, though the chromatin in the same cell stains black. It is interesting to note that the spireme and chromosomes in tissue so treated have an unstained core surrounded by darkly staining chromatin just as if the nucleolus furnished the material for their central regions. When fixed with $Li_2Cr_2O_7$, the nucleolus is no longer spherical but shaped like *Amoeba radiosa* with pointed 'pseudopods' extending out to the periphery of the nucleus. The meaning of this fixation image, so far unique, is not at all clear.

Chromatin is fixed only by acid solutions. This is what we would expect from the work of Kuwada and Sakamura (11) who found that unfixed chromosomes were dissolved by a mixture of $K_2C_2O_4$ and $H_2C_2O_4$ when the pH was as alkaline as 5·6, and from the work of Earl (6) who found that chromosomes fixed with chrom-acetic acid were dissolved from slides immersed in tribasic sodium phosphate when the alkalinity of the solution reached pH 5·0. Chromatin seems to be a salt of nucleic acid and a protein base (Mathews 17). The protein seems to be fundamentally basic, thus it unites with the anion and when the solution is not sufficiently acid to destroy the nuclear proteins, it is not fixed, but dissolved either by the fixing fluid or by the succeeding washing and dehydration.

The nuclear lymph, on the contrary, acts as an acid, for it is soluble in all acid chromates and is fixed by all basic ones with the single exception so far of $Li_2Cr_2O_7$. It even seems to permeate the chromatin, for when it is fixed, even though the chromatin is dissolved, the nuclei are solid and show no vacuoles where the chromatin had been. If the nuclear lymph were composed primarily of nucleic acid, its characteristic behavior during fixation would be expected, although there is no justification for assuming it to be so composed merely from the evidence here presented.

NAYLOR (18) found an important difference in the staining reaction of resting chromatin and of chromatin in dividing cells. Thus when sections of hyacinth root tips, fixed in chrom-acetic acid, were stained in a mixture of methyl blue and safranin and washed with a buffer mixture of pH 5·1, the resting chromatin united with the colored anion and the chromosomes and nucleoli with the colored cation. In the present investigation, likewise, a difference in the fixation of resting and dividing chromatin was found. As the fixing fluid grows more alkaline, the resting chromatin is dissolved before the chromosomes. Thus in a mixture of lead and ammonium bichromate at pH 4·6 no chromatin reticulum is found in resting nuclei but certain cells show well delineated chromosomes at the equatorial plate. Copper bichromate (pH 4·6) fixes the spireme and chromosomes but not the resting chromatin. Zinc bichromate (pH 5·2) fixes no chromatin except that in the periphery of the chromosomes at metaphase. It is perhaps significant that this change in the reaction of the chromatin should occur in those mitotic phases where the nucleolus disappears as a distinct cell organ. NAYLOR's (18) plate shows that the chromatin acquires the staining reactions of the nucleolus as the latter disappears.

Mitochondria are fixed only by solutions of bichromates more basic than 4·2 pH, with the exception of silver ammonium bichromate (pH3·0). Making the same assumption as to the nature of this fixation of the various nuclear elements, it would seem that the mitochondria behave primarily as acids and are fixed by uniting with the cation of the fixing fluid. There is nothing in these findings which would indicate that mitochondria do not also contain phospho-lipins or lecithin-like substances. As the reactions of mitochondria and chromatin to chrome fixation are so different, the former appearing in the basic fixations and the latter in the acid, there should be no longer any confusion of mitochondria with any "chromidial substance" extruded from the nucleus.

Once the critical pH is passed, a considerable increase in the alkalinity of the fixative seems to have but little influence upon either the shape, size or staining properties of the mitochondria. We cannot be sure of this, however, until more is known about the rate of penetrations of the various fixatives, for at present there is no guarantee that the tissue is actually killed and fixed at the exact pH of the fixative. Other cations beside hydrogen, however, seem to influence the form of the fixed mitochondria and even seem to influence the pH at which fixation will occur. Thus mitochondria are fixed by $(NH_4)_2Cr_2O_7$ at pH 4·2, but not by $Li_2Cr_2O_7$, $CuCr_2O_7$, $GlCr_2O_7$, etc. The influence of the various cations upon the form the fixed mitochondria take is interesting, but its interpretation is by no means obvious. Thus fixed with ammonium bichromate the mitochondria are long narrow threads, while the bichromates of potassium and sodium fix them as shorter, stouter threads and slender rods; copper, glucinum and cerium fix them as rods, while zinc, cadmium and alkaline earths tend to break them up into chains of granules. Lithium causes them to appear as prolate spheroids. One should be cautious, however, in interpreting these changes of shape. Mitochondria in living cells are altered by changes in pH and until it is kown at what pH the cells are actually fixed, the amount of this change of form due merely to the hydrogen-ion concentration cannot be determined.

As the alkalinity of a bichromate solution is increased, it reaches a point where the fixation image changes from the acid to the basic. Generally this change is sudden and complete. As the chromatin disappears, the nuclear lymph and the mitochondria are fixed. At times, though, the change will occur in some cell elements before others. In tissue fixed with $(NH_4)_2Cr_2O_7$, at the critical point there are always certain cells whose chromatin is dissolved and whose nuclear lymph is fixed but whose mitochondria are not. In general, the change is sudden with the alkali elements, less sudden with the alkali earths, while with certain elements as cations, the two fixation images overlap. Copper bichromate (pH 4·6) always fixes dividing chromatin and nuclear lymph and generally the mitochondria. The addition of 1% copper sulphate causes it to fix all cell elements except resting chromatin. Glucinum behaves as does copper, always fixing dividing chromatin and generally the mitochondria. Cerium bichromate (pH 4·8) regularly fixes mitochondria, nuclear lymph and dividing chromatin. Zinc bichromate (pH 5·2) fixes mitochondria and nuclear lymph; while the only chromatin it fixes is in the periphery of chromosomes at metaphase. Certain mixed bichromates also preserve elements of both acid and basic fixation. Lead-ammonium bichromate (pH 4·4) fixes mitochondria, nuclear lymph and chromosomes at metaphase. Silver-ammonium bichromate (pH 3·0) gives the usual acid fixation image and in addition preserves some mitochondria in the central cylinder, though they are obscured somewhat by the disorganized cytoplasm.

Certain cytoplasmic elements are fixed by both acid and basic solutions. The two images, though, are quite distinct. Acid fixatives make the cytoplasm appear disorganized, streaked and washed out, while with basic fixatives it is fixed smoothe and solid except for well delineated vacuoles whose growth and development can easily be followed from the apex of the root tip back through the region of elongation.

That the first reaction between a chromate fixative and the tissue fixed is a double decomposition with the cation of the fluid uniting with certain elements of the tissue and the anion with certain other elements, is an hypothesis which would explain the effects of pH upon the fixation image. It does not, however, account for all of the phenomena connected with chrome fixation. If copper bichromate (pH 4·6) fixation consists merely of the copper uniting with the nucleolus, nuclear lymph, and mitochondria, and the bichromate radical uniting with the nucleolus and chromatin, it would be expected that as long as the pH was kept constant the substitution of a sulphate radical for the bichromate would not prevent the copper uniting with the tissue, provided the sulphate radical itself did not obstruct fixation. Copper sulphate mixed with copper bichromate does not disorganize the tissue but actually improves fixation, but a 2% solution of copper sulphate (pH 4·6) by itself is not even a poor fixing fluid; cells treated with it are merely a jumble of unrecognizable fragments. That this disorganization is not due to the sulphate ion being the only anion in the fixing fluid is shown by the fact that a mixture of copper sulphate and chromic sulphate (pH 4·6) is an excellent fixative for nuclei, and with the addition of 4% formalin fixes both chromosomes and mitochondria. Thus chrome fixation can be brought about by compounds in which chromium is a cation and the only anion is sulphate.

Frequently tissue fixed with a bichromate and stained dark brown by the bichromate ion will have become a bright green before it is completely dehydrated, showing that the brown chromate ion has been reduced to the green chromic one. That chrome fixation must be in part an oxidizing process is rendered quite probably by the fact that the bichromates are oxidizing agents and are reduced by the process of fixation. This process seems to be a form af chrome tanning. In the two bath processes of tanning, the chromate salt is reduced to a chromic one in which condition it combines with the skin proteins yielding a very stable leather (WILSON 21). It should be recorded, however, that although chrome tanned leather can be untanned with Rochelle salts (WILSON), root tips fixed in copper bichromate and subsepuently soaked in Rochelle salts are not appreciably "unfixed", even though apparently all of the bichromate ions have been removed.

If we postulate that the first reaction in chrome fixation is a double decomposition and recombination of the anions and cations of the fixative with the proteins of the tissue, we can account for the know facts by postulating that there is in addition an oxidation-reduction reaction resulting in chromium being held in the tissue both as an anion and as a cation. A mixture of copper bichromate and chromic sulphate (pH 4·6) preserves all of the structures fixed by either the acid or basic fluids. UNNA (20) held that chromium was present in the tissue in the form of a chromic chromate.

While the observed facts of chrome fixation are explained by the assumption that the reaction consists first of a double decomposition and combination of the cations with certain cell elements, and the anions with others, followed by a reduction of the chromium so that it exists both as anion and cation and thus forms stable compounds with all of the tissue fixed, there is no reason to assume that these are the only reactions which occur when chromium is used in any of the usual complex fixatives. The presence of other chemicals, often in relatively minute quantities, greatly influences and often apparently alters the nature of the fixation. Osmium tetroxide mixed with chromic acid, as in FLEMMING's without acetic acid, will fix mitochondria in a much more acid solution than will any bichromate.

Formalin also greatly modifies chrome fixation, for its presence in a bichromate solution will cause the fixation of mitochondria at an acidity when they would not be fixed by the bichromate alone. Mitochondria have been fixed by mixtures of formalin and $Cr_2(SO_4)_3$ as acid as pH 2·0. Chromic sulphate and copper oxide at pH 4·6 give an excellent acid fixation image. The addition of 4% formalin, even if the pH is held constant, changes the image to basic. The addition of formalin here not only causes the fixation of the mitochondria, it also prevents the fixation of resting chromatin. Any interpretation of the influence of formalin in the mixtures must wait until it is determined whether it acts as an oxidizing or a reducing agent. In contact with a bichromate it is a reducer, but it has been suggested that it acts as an oxidizer when in contact with the cell proteins (KINGSBURY 10).

The addition of a little copper acetate to a solution of copper bichromate has a great influence on the fixation image, even though the pH is held constant at 4·6. Thus if the solution contains 2·5% copper bichromate, the fixation image will be shifted to a purely acid one if as much as ·2% of acetic acid be added, even in the presence of an excess of CuO. As much as ·1% acetic acid will cause the resting chromatin to be fixed. In as much as the chromate and acetate radicals are generally mixed in the usual fixing fluids, there is a present need for an investigation of the exact effects of the acetate ion upon chrome fixation.

The fact that small amounts of such substances as ammonium, formalin and acetates when mixed with chromium compounds alter the fixation image so markedly, need not mean that they participate directly in the reactions of the chromium. Evidence has been cited to show that the first toxic substance in a fixation mixture to penetrate the tissue seemingly determines the fixation image. These substances all penetrate extraordinarily rapidly, and possibly fix the tissue before the compounds of chromium reach the scene of action. The chromatization would then tend to perpetuate the structure fixed. This would explain why the interior of a root tip fixed with a mixture of ammonium and silver bichromate (pH 4·4) would have the fixation image of ammonium; why the interior fixed with chromic sulphate and formalin would have the image of a formalin fixation; and why a trace of copper acetate should alter the fixation image of copper bichromate. It would also account for certain very interesting results of MASCRÉ (15).

MASCRÉ found that while monochloracetic acid (3%), cyanacetic (3%) and trichloracetic (2%) alone would destroy mitochondria, mixed with 20% formalin they would preserve them so that postchromatization was unnecessary. Acetic acid (5%), however, even so mixed, destroyed all mitochondria in spite of the fact that in the solutions used it was less acid than the others. (Monochloracetic pH 1·6, cyanoacetic pH 1·3, trichloracetic pH 0·6—0·7, acetic pH 1·9—2·0). If the acetic acid penetrated more rapidly than the formalin and the formalin more rapidly than the other acids, the above results would be expected. While CROZIER (5) has shown that in very dilute solutions the chloracetic acids penetrate *Chromodorus* tissue more rapidly than acetic acid, it by no means follows that they would do so in the solutions here used. The order of the rates of penetration of these acids are dependent upon the concentrations (CROZIER 5), and in relatively concentrated solutions the order of penetration of acids is often reversed. In fact, by giving formalin a head start, a mixture of acetic acid and formalin can fix mitochondria.

SUMMARY

The fixation image of a bichromate depends upon the pH at which it is used. If the solution is more acid than a given critical point, the image will be practically that of chromic acid, i. e. in resting cells the nucleolus will be a darkly staining globule in the center of a hollow nucleus whose periphery is formed by a chromatin reticulum. No mitochondria will be preserved and the cytoplasm will be disorganized. In dividing cells, the chromosomes and spindle fibers will be well fixed. If the solution is on the alkaline side of the critical point, the fixation image will be quite different. In the resting stage the nucleolus is fixed as in the acid fixative, but here it appears in a solid nucleus composed of fixed nuclear lymph. The chromatin and spindle fibers will be dissolved so the tissue will show no mitotic figures. The mitochondria and cytoplasm will be well fixed. The change from one fixation image to the other is as a rule sudden and complete, the point of change depending upon the bichromate used and ranging among those investigated from pH 4·2 in the case of ammonium to about pH 5·2 in the case of zinc. After the change has taken place, a further change in pH has little effect upon the fixation image. With an excess of the cation present as an oxide, hydroxide or carbonate, however, certain bichromates buffer at points where the two fixation images overlap. Thus copper (pH 4·6), glucinum (pH 4·6) and cerum (pH 4·8) bichromate fix nucleoli, nuclear lymph, dividing chromatin, spindle fibers, mitochondria and cytoplasm. The bichromates of barium, bismuth, iron, lead, aluminum and mercury are too acid to give any but the acid fixation image. Mercury prevents the nucleolus from staining with HAIDENHAIN's haematoxylin. Ammonium (pH 4·2), sodium (pH 4·4) and potassium (pH 4·4) bichromate, in the absence of free chromic acid, give only the basic fixation image. Strontium (pH 5·6), calcium (pH 5·6), magnesium (pH 6·2) and cadmium (pH 5·6) bichromate buffer at points where their fixation image is basic.

Zinc (pH 5·2) gives a basic fixation image except that greatly swollen chromosomes are fixed at metaphase. A mixture of silver and ammonium bichromate (pH 3·0) gives the usual acid fixation image and in addition mitochondria in badly disorganized cytoplasm. Lithium bichromate (pH 4·6) gives the basic fixation image in the epidermal cells and somewhat disorganizes the interior layers; and in more basic solution gives a fixation image which is as yet unique. Chromic sulphate buffered with copper oxide at pH 4·6 gives an acid fixation image; with formalin added the pH is not changed but the image becomes basic. Slight traces of the acetate ion will shift a basic fixation image to an acid one even if the pH is held constant. Such substances as acetates, ammonium salts and formalin, which greatly alter the fixation image when mixed with chromium compounds, probably do not participate directly in chrome fixation but, because of their ability to penetrate very rapidly, actually fix the tissue before the chromium reaches the scene of action, the chromium constituents serving merely for a post-chromatization.

The form of the mitochondria depends upon the cation of the bichromate fixatives as well as that of the pH. Fixed with ammonium bichromate, they are long slender threads occasionally forming a reticulum. Sodium and potassium bichromate fix them as coarser threads and slender rods; copper, glucinum and cerium as shorter, stouter rods; while with lithium they fix as prolate spheroids. Zinc, cadmium and the alkali-earths as cations tend to fix the mitochondria, especially in the region of elongation, as rods and chains of granules. Chromic sulphate and formalin fix them as slender threads.

The findings of the present investigation are best explained by assuming that chrome fixation consists first, of a double decomposition in which the cation of the fixative unites with certain elements of the cell and the anion with others; second, followed by a reduction of part of the chromate to a chromic salt, the chromium then being combined with the tissue both as anion and cation. Chrome fixation can be considered a form of chrome tanning.

The Author wishes to thank Professor E. J. Cohn and Dr. G. A. Alles for their helpful criticism. Especially are his thanks due to Professor E. M. East, in whose laboratory the work was done, for his interest, assistance and most helpful criticism.

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APPENDIX

The following fixatives have been tested thoroughly on the root tips of Zea and may be useful in fixing other tissue. Fixation in every case should last from 36 to 48 hours. Wash 1 hour in running water and dehydrate an hour each in the following percentages of alcohol: 5, 11, 18, 30, 50, 70 (over night), 85, 95, 100, 100. Any grade of alcohol should be changed if it grows murky. Mordant in 4 % Iron Alum (pH 3·0) for 2 hours and stain in .5 % HAIDENHAIN's haemotoxylin for a like time. Destain in 1 % Iron Alum under the microscope. Destaining should never proceed as far in tissue fixed to show mitochondria as in tissue fixed to show nuclei.

1.	Chromic sulphate $Cr_2(SO_4)_3 \cdot 15\ H_2O$	5·	grams
	Copper oxide CuO)	·5	„
	Water	100·	c. c.

pH 4·6

This fixes all stages of the nuclei and does not disrupt the cytoplasm too much. Mitochondria destroyed.

2.	Chromic sulphate	5·	grams
	Copper oxide	·5	„
	Formalin (40 % Formaldehyde)	10·	c. c.
	Water	90·	„

pH 4·6

This fixes the mitochondria in the interior of the tissue and preserves the chromatin in all stages except in the resting nucleus. It preserves the cytoplasm so that the growth and development of vacuoles can be observed.

3.	$CuCr_2O_7$	2·5	grams
	CuO	·5	„
	$CuSO_4$	1·	„
	Water	100·	„

pH 4·6

This preserves mitochondria, nuclear lymph and dividing chromatin.

4.	CrO_3	2·5	grams
	$HC_2H_3O_2$	2·5	c. c.
	CuO	slight excess	
	Water	100·	c. c.

pH 4·6

This fixes the nuclei at all stages and particularly the nucleoli. It destroys all mitochondria.

5.	CrO_3	2·5	grams
	$GlCO_3$	slight excess	
	Water	100.	c. c.

pH 4·8

This gives a fixation image very much as No. 3.

6.	CrO_3	2·5	grams
	$Ce_2(CO_3)_3$	slight excess	
	Water	100·	c. c.

pH 4·8

This is a very reliable fixing fluid for both chromosomes and mitochondria.

7.	$CuCr_2O_7$	1·5	grams
	$Cr_2(SO_4)_3$	2·5	„
	CuO	·5	„
	Water	100·	c. c.

pH 4·6

This gives a fixation image like No. 6.

8. $K_2Cr_2O_7$	1·25	grams
$(NH_4)_2Cr_2O_7$	1·25	„
$CuSO_4$	1·	„
Water	100·	c. e.

pH 4·4

This modification of Erliki's fixative is a very reliable one for fixing mitochondria. It destroys all chromatin.

DESCRIPTION OF PLATES

These photographs were taken with a Leitz "Makam", camera. None are retouched. The magnification is 800 times. The stain used was iron-alum haematoxylin. No counter stain was used. Unless otherwise stated, the tissue is taken from the root tip of *Zea maize.*

Plate V

Fig. 1. Longitudinal section of root cap of Zea fixed with 2% H_2CrO_4. Note shrinkage of protoplast and staining of starch grains.

Fig. 2. Fixed with $CuCr_2O_7$ (pH 4·6). Both dividing chromatin and mitochondria are fixed.

Fig. 3. Fixed with $CuCr_2O_7$ and $Cu(C_2H_7O_2)_2$ (pH 4·6). Note hollow resting nuclei and nucleolus knocked out of nucleus.

Fig. 4. Fixed with H_2CrO_4. Note macerated appearance of tissue and hollow nuclei.

Fig. 5. Fixed with $K_2Cr_7O_2$ (pH 4·4). Note mitochondria, fixed nuclear lymph and absence of all chromatin.

Fig. 6. Fixed as Fig. 5. Cells in region of elongation showing two sitzes of mitochondria; the larger ones are developing into plastids.

Plate VI

Fig. 7. Fixed as Fig. 3. Showing 2 nucleoli knocked out of nuclei and partially out of the cells.

Fig. 8. Fixed as Fig. 7. Nucleolus shown attached to spireme.

Fig. 9. Fixed with $Li_2Cr_2O_7$ (pH 6·0) showing swollen nuclei, spherical mitochondria and amoeboid shaped nucleoli.

Fig. 10. Same as Fig. 9.

Fig. 11. Fixed with Cerium bichromate (pH 4·8) showing both dividing chromatin and mitochondria.

Fig. 12. Fixed with $GlCr_2O_7$ (pH 4·6) showing both dividing chromatin and mitochondria.

Fig. 13. Fixed with Iron bichromate (pH 2·0) showing hollow nuclei and sligthly shrunken protoplast.

Plate VII

Fig. 14. Fixed with $HgCr_2O_7$ (pH 2·0) showing hollow nuclei with lightly staining nucleoli.

Fig. 15. Fixed with $(NH_4)_2Cr_2O_7$ (pH 4·2) showing thredlike mitochondria and absence of all chromatin.

Fig. 16. Fixed with $MgCr_2O_7$ (pH 6.2) showing mitochondria in the form of granules and chains of granules.

Fig. 17. Same as Fig. 15.

Fig. 18. Fixed with $CaCr_2O_7$ (pH 5·6) showing granular mitochondria.

Fig. 19. Fixed with a mixture containing NH_4^+, Ag^+, $Ag(NH_3)_2^+$, $-Cr_2O_7$ and $-NO_3$ (pH 4·4). The cells on the left are near the periphery and show the acid fixation image; those on the right are nearer the center and show the basic.

Plate VIII

Fig. 20. Fixed with $CdCr_2O_7$ (pH 5·6) showing typical basic fixation images except for traces of disorganized dividing chromatin.

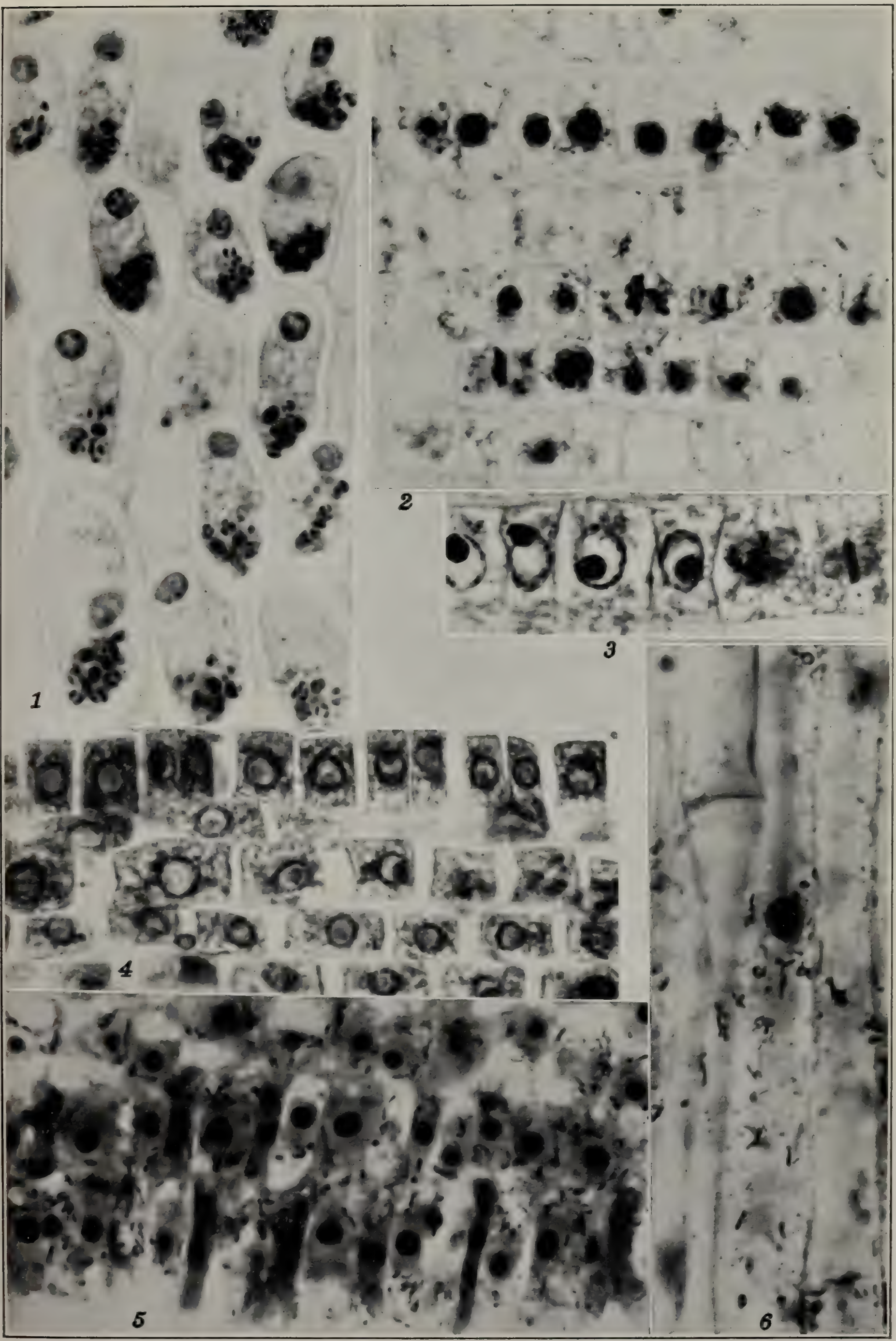
Pig. 21. Fixed with $ZnCr_2$ (pH 5·2) showing basic fixation image except for hollow chromosomes at metaphase.

Fig. 22. Fixed $Cr_2(SO_4)_3$, $CuSO_4$ and Formalin (pH 4·6) showing dividing chromating, mitochondria and hyaloplasm.

Fig. 23. Same as Fig. 22, except for the formalin, showing typical acid fixation image.

Fig. 24. Fixed with $SrCr_2O_7$ (pH 5·6). Basic fixation image.

Fig. 25. Fixed with K_2CrO_7 (pH 4·4) showing mitochondria in epidermal layer and plastids in cortex.



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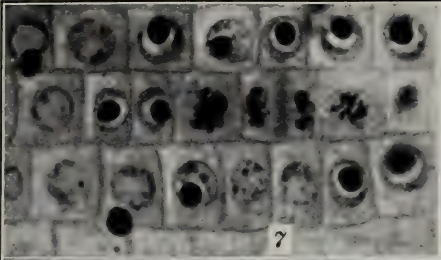
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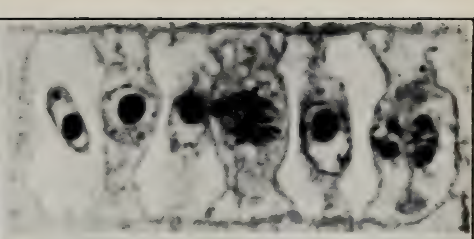
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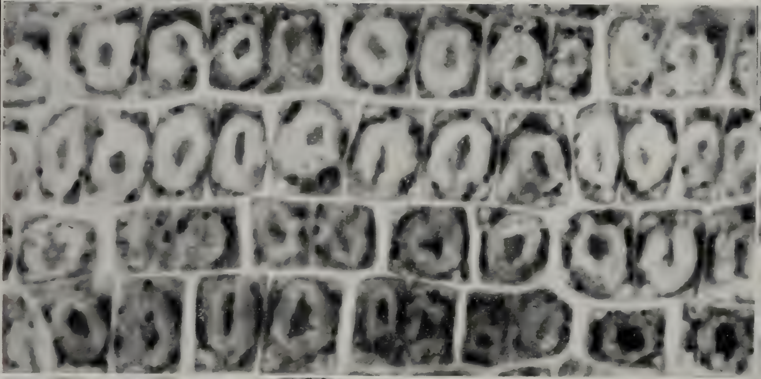
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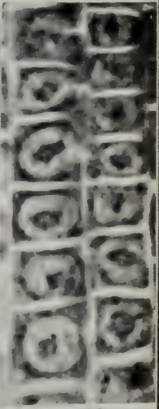
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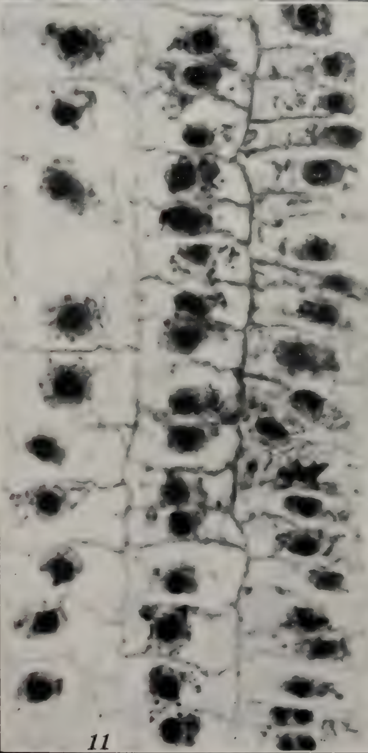
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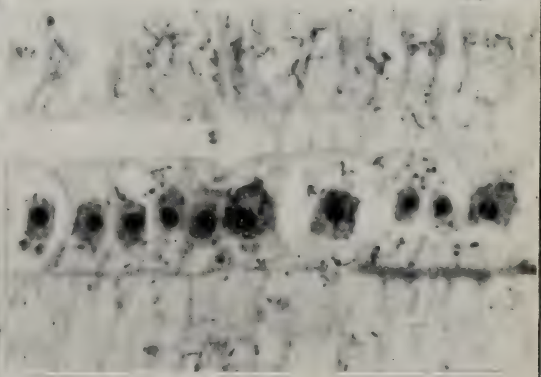
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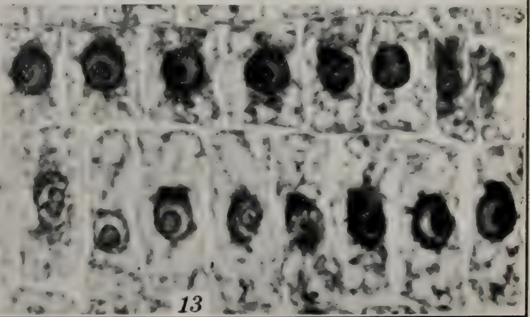
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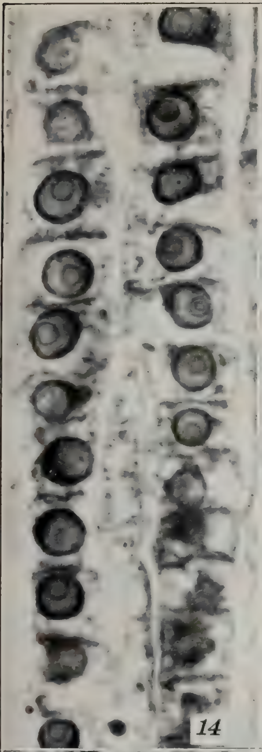
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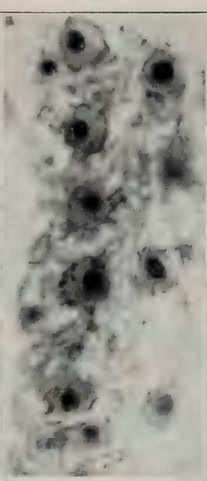
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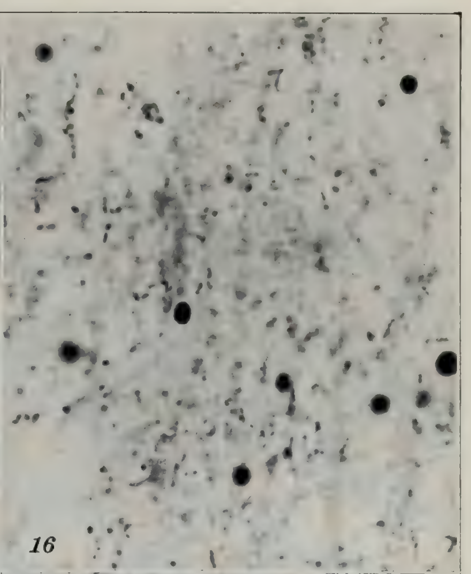
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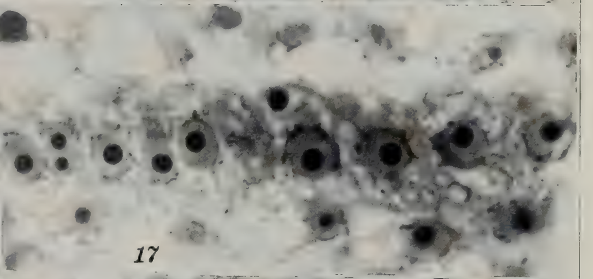
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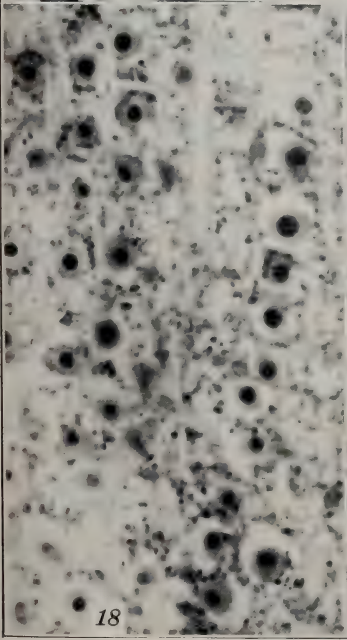
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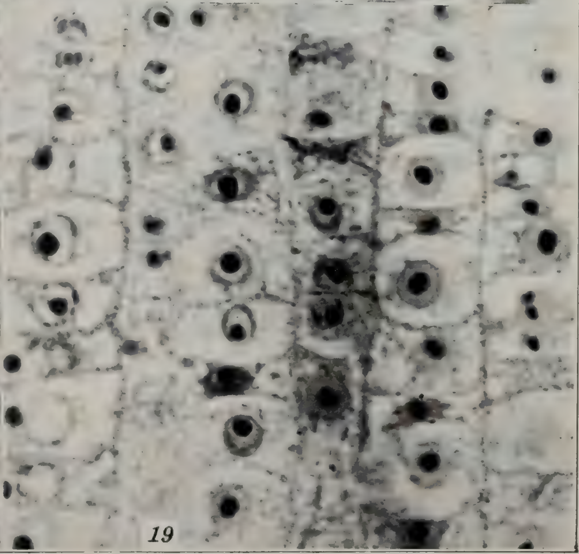
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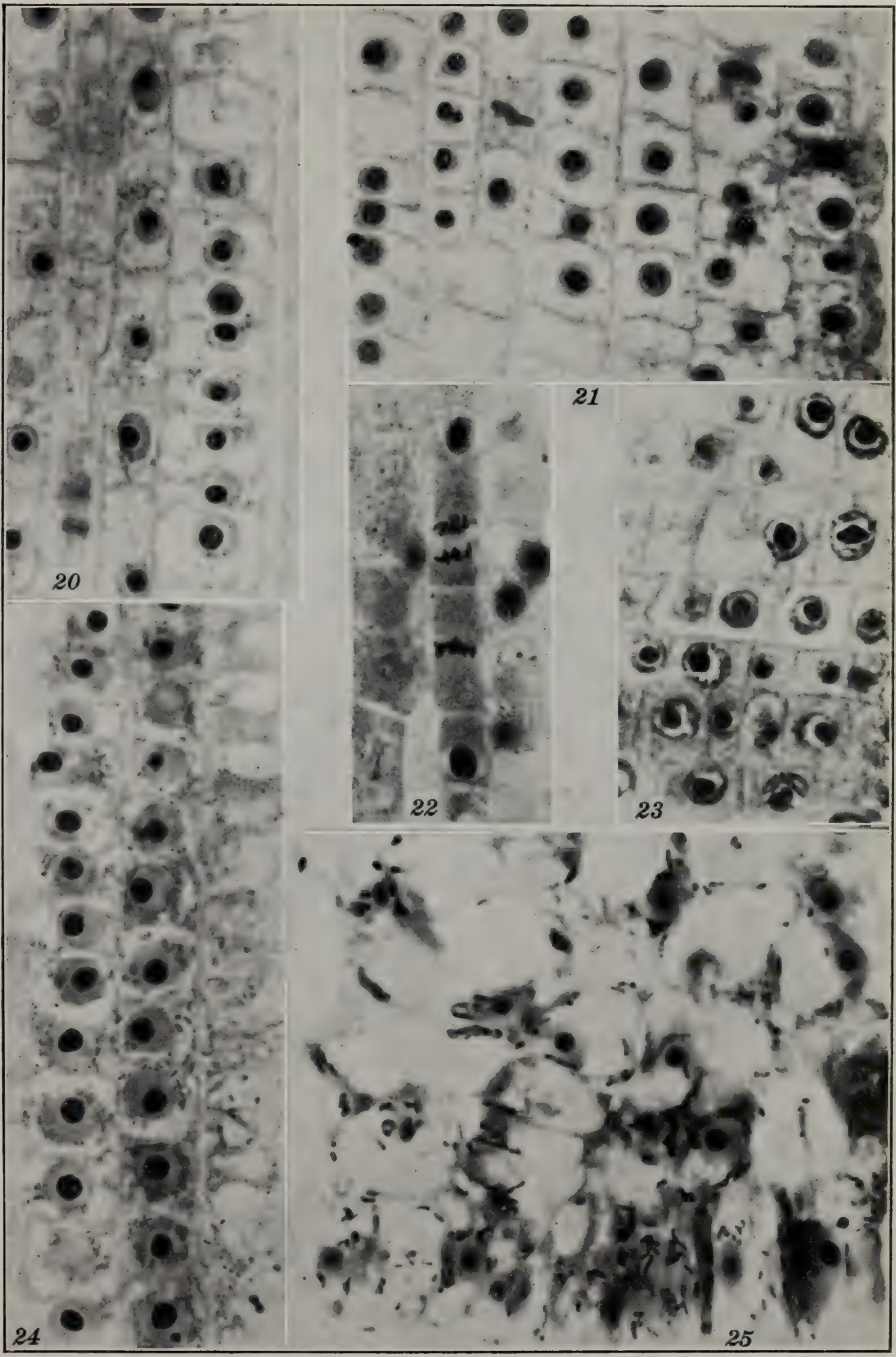

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Zeitschrift für induktive Abstammungs-
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E. M. East:

The Genetics of Trimorphism in Lythrum salicaria

The Genetics of Trimorphism in Lythrum salicaria

E. M. East

Bussey Institution of Harvard University, Boston, Mass.

Genetic studies of *Lythrum salicaria* L. have been carried on at the Bussey Institution of Harvard University since 1920 as a part of an extended series of investigations on self-sterility. The plants are not completely self-sterile, since all three types will produce some seed after self-pollination; but the self-fertility exhibited is of low grade. The Mid styled plants are the most fertile, the resulting progeny consisting of Mids and Longs. Long styled plants are less fertile, the progeny consisting only of Longs. I have obtained no progeny from Short styled plants, but Darwin and Mrs. Barlow obtained all three forms from selfed shorts.

It will be recalled that the sporangia in this species are arranged in three tiers and that matings between gametes borne in sporangia of the same tier, the so-called "legitimate" matings of Darwin, are much more fruitful than matings of any other type, — the "illegitimate" unions of Darwin. I have been unable to make a satisfactory interpretation of this self- and cross-sterility in either physiological or genetic terms. I can only say that the genes which condition the form of flower appear to be distributed in the same manner to the three tiers of sporangia of each plant. In other words, "legitimate" and "illegitimate" pollinations yield similar results. Kostoff[1]) has shown that in the cross Mid style × Long style pollen-tube growth is much slower after an "illegitimate" pollination than it is after a "legitimate" pollination. But it is uncertain whether similar growth rates hold for all "illegitimate" and "legitimate" pollinations. Nor is it known what

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*) Dontcho Kostoff. Pollen-tube growth in *Lythrum salicaria*. Proc. Nat. Acad. Sci., 13: 253—255, 1927.

relation the pollen-tube growth curves formed by pollen bearing fat and starch respectively as reserve food materials hold to each other. I have not yet been able to convince myself that there is a consistent difference in growth rate between pollen tubes of these two types.

The inheritance of the flower form, however, yields to a simple interpretation. The flower differences are conditioned by three genes. The Long styled flower is the triple recessive. The Short styled flower is determined by a gene A which is probably lethal in the homozygous condition. Short styled flowers may or may not carry the genes which determine the Mid style type. Mid styled flowers are determined by apparently duplicate genes M_a and M_b carried in the same chromosome. In both the male and the female gametes there is about 10 per cent crossing-over, with perhaps slightly less crossing-over in the females than there is in the males. The presence in the homozygous condition of either or both of the factors determining Mid produces a lethal effect. The genetic constitutions of the theoretically possible types are shown in Table I. It has not been proved that either M_a or M_b can exist alone, though *a priori* this seems probable. Adequate proof[1]) of the remaining

Table I

Genetic Constitution of Plants having Different Style Lengths

Constitution	Type
$a\ m_a - m_b \cdot a\ m_a - m_b$	Long style
$a\ M_a - M_b \cdot a\ m_a - m_b$	Mid style
$a\ M_a - m_b \cdot a\ m_a - M_b$	,, ,,
$a\ M_a - m_b \cdot a\ m_a - m_b$	,, ,,
$a\ m_a - M_b \cdot a\ m_a - m_b$	,, ,,
$A\ M_a - M_b \cdot a\ m_a - m_b$	Short style
$A\ M_a - m_b \cdot a\ m_a - M_b$	,, ,,
$A\ M_a - m_b \cdot a\ m_a - m_b$	,, ,,
$A\ m_a - M_b \cdot a\ m_a - m_b$	,, ,,
$A\ m_a - m_b \cdot a\ m_a - m_b$	,, ,,

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[1]) Cf. E. M. East. Inheritance of trimorphism in *Lythrum salicaria*. Proc. Nat. Acad. Sci. 13: 122—124, 1927.

postulates exists, including evidence of high and of low seed formation and seed viability in crosses where high lethal effects and where low lethal effects are expected.

The cross-over percentage was calculated from selfing Mid-styled plants of constitution $M_a \text{ — } M_b \,.\, m_a \text{ — } m_b$. The actual numbers obtained were 194 Mids: 75 Longs, as shown in Table II. The crossover calculation was checked with other data, but it cannot be maintained that it is as accurate as might be desired. I hope to make a new determination based upon larger numbers at the end of the 1927 season.

Table II

Theoretical Results from Selfing Mid-styled Plants of Constitution $M_a \text{ — } M_b \cdot m_a \text{ — } m_b$

Homozygotes lethal and cross-overs 10 per cent

Mids	$M_a \text{ — } M_b \cdot m_a \text{ — } m_b$	form	57.7	per	cent	progeny
,,	$M_a \text{ — } m_b \cdot m_a \text{ — } m_b$	,,	6.4	,,	,,	,,
,,	$m_a \text{ — } M_b \cdot m_a \text{ — } m_b$	,,	6.4	,,	,,	,,
,,	$M_a \text{ — } m_b \cdot m_a \text{ — } M_b$	,,	0.4	,,	,,	,,
	$m_a \text{ — } M_b \cdot M_a \text{ — } m_b$	,,	0.4	,,	,,	,,
Longs	$m_a \text{ — } m_b \cdot m_a \text{ — } m_b$	,,	28.8	,,	,,	,,
Actual numbers 194 Mids : 75 Longs						

Table III

Theoretical Results from Selfing Mid-styled Plants of Constitution $M_a \text{ — } m_b \cdot m_a \text{ — } M_b$

Homozygotes lethal and cross-overs 10 per cent

Mids	$M_a \text{ — } m_b \cdot m_a \text{ — } M_b$	form	80.6	per	cent	progeny
,,	$M_a \text{ — } m_b \cdot m_a \text{ — } m_b$	,,	9.0	,,	,,	,,
,,	$m_a \text{ — } M_b \cdot m_a \text{ — } m_b$	,,	9.0	,,	,,	,,
,,	$M_a \text{ — } M_b \cdot m_a \text{ — } m_b$	,,	1.0	,,	,,	,,
Longs	$m_a \text{ — } m_b \cdot m_a \text{ — } m_b$	,,	0.5	,,	,,	,,
Mids of type $M_a \text{ — } m_b \cdot m_a \text{ — } m_b$ selfed give ratio of 2 Mids : 1 Long						

As yet it has been impossible to distinguish Mids of type $M_a \text{ — } M_b \cdot m_a \text{ — } m_b$ from Mids of types $M_a \text{ — } m_b \cdot m_a \text{ — } m_b$ or $m_a \text{ — } M_b \cdot m_a \text{ — } m_b$. There is not a great deal of difference in the results to be expected after selfing these three types, however; the first yielding 71.2 Mids: 28.8 Longs, the second and third yielding 66.7 Mids : 33.3 Longs. These ratios are in marked contrast to the ratio to be expected when Mids of constitution $M_a - m_b . m_a - M_b$ are selfed, as shown in Table III. This ratio is 99.5 Mids : 0.5 Longs.

Since it is not easy to obtain satisfactory ratios from selfed plants, a mixed lot of Mids were crossed with pollen from two plants of constitution $M_a - m_b . m_a - M_b$. The ratios to be expected from such crosses are shown in Table IV. From their derivation the Mids used as females should be preponderantly of the first type or of the first three types, with a few of the fourth type. One might logically expect, therefore, a ratio of about 3 per cent Longs. The actual results were 2.8 per cent Longs in a total of 642 plants.

Table IV

Theoretical Results Obtained when Various Mids are Crossed with Mid of Constitution. $M_a - m_b \cdot m_a - M_b$

$M_a - M_b \cdot m_a - m_b \times M_a - m_b \cdot m_a - M_b = 95.7$ M : 4.3 L
$M_a - m_b \cdot m_a - m_b \times M_a - m_b \cdot m_a - M_b = 96.7$ M : 3.3 L
$m_a - M_b \cdot m_a - m_b \times M_a - m_b \cdot m_a - M_b = 96.7$ M : 3.3 L
$M_a - m_b \cdot m_a - M_b \times M_a - m_b \cdot m_a - M_b = 99.5$ M : 0.5 L
A mixed series of such crosses gave 624 M : 18 L = 97.2 M : 2.8 L

When Mids are crossed with Longs, again there is a great contrast in the results to be expected when Mids of constitution $M_a - m_b \cdot m_a - M_b$ are compared with Mids of other constitutions. As shown in Table V, three types of Mids give very nearly 1 Mid : 1 Long. The actual results

Table V

Mids crossed with Longs

$M_a - m_b \cdot m_a - m_b \times m_a - m_b \cdot m_a - m_b = 50$ M : 50 L
$m_a - M_b \cdot m_a - m_b \times m_a - m_b \cdot m_a - m_b = 50$ M : 50 L
$M_a - M_b \cdot m_a - m_b \times m_a - m_b \cdot m_a - m_b = 55$ M : 45 L
Obtained 229 M : 262 L

$M_a - m_b \cdot m_a - M_b \times m_a - m_b \cdot m_a - m_b = 95$ M : 5 L
Obtained 494 M : 42 L = 92 M : 8 L

were 229 Mids : 262 Longs. The other cross should give 95 Mids : 5 Longs. The ratio found was 494 Mids : 42 Longs, that is to say, 92 Mids : 8 Longs.

When Shorts are crossed with Longs, various results are to be expected; but they fall readily into three groups, as shown in Table VI.

Table VI

Shorts crossed with Longs

$A\,m_a - m_b \cdot a\,m_a - m_b \times a\,m_a - m_b \cdot a\,m_a - m_b =$ 1.0 L : 0.0 M : 1.0 S

Obtained 437 L : 481 S

$A\,M_a - m_b \cdot a\,m_a - m_b \times a\,m_a - m_b \cdot a\,m_a - m_b =$ 25.0 L : 25.0 M : 50.0 S

$A\,m_a - M_b \cdot a\,m_a - m_b \times a\,m_a - m_b \cdot a\,m_a - m_b =$ 25.0 L : 25.0 M : 50.0 S

$A\,M_a - M_b \cdot a\,m_a - m_b \times a\,m_a - m_b \cdot a\,m_a - m_b =$ 22.5 L : 27.5 M : 50.0 S

Obtained 95 L : 115 M : 225 S = 21.8 L : 26.4 M : 51.8 S

$A\,M_a - m_b \cdot a\,m_a - M_b \times a\,m_a - m_b \cdot a\,m_a - m_b =$ 2.5 L : 47.5 M : 50.0 S

Obtained 9 L : 77 M : 116 S = 4.4 L : 38.1 M : 57.5 S

First there is the Short which does not carry Mid, with the constitution $A\,m_a - m_b \,.\, a\,m_a - m_b$. Theoretically reciprocal crosses with Longs should yield a ratio of 1.0 Short : 1.0 Long. Actually there were obtained 481 Short : 437 Long. Three other types of Short should give ratios approaching 2 Shorts: 1 Mid : 1 Long. The data obtained were 225 Short : 115 Mid : 95 Long, or 51.8 Short : 26.4 Mid : 21.8 Long. Quite a different ratio is to be expected when Shorts of constitution $A\,M_a - m_b \,.\, a\,m_a - M_b$ are used in crosses with Longs. Here 50 Shorts: 47.5 Mids : 2.5 Longs are to be anticipated. The actual data were 116 Shorts : 77 Mids : 9 Longs, or 57.5 Shorts : 38 Mids : 9 Longs.

Table VII

Mids Crossed with Shorts which are known not to carry Mid factors.

$a\,M_a - m_b \cdot a\,m_a - m_b \times A\,m_a - m_b \cdot a\,m_a - m_b =$ 25.0 L : 25.0 M : 50.0 S

$a\,m_a - M_b \cdot a\,m_a - m_b \times A\,m_a - m_b \cdot a\,m_a - m_b =$ 25.0 L : 25.0 M : 50.0 S

$a\,M_a - M_b \cdot a\,m_a - m_b \times A\,m_a - m_b \cdot a\,m_a - m_b =$ 22.5 L : 27.5 M : 50.0 S

Obtained 126 L : 125 M : 243 S = 25.3 L : 25.5 M : 49.2 S

$a\,M_a - m_b \cdot a\,m_a - M_b \times A\,m_a - m_b \cdot a\,m_a - m_b =$ 2.5 L : 47.5 M : 50.0 S

Obtained 5 L : 36 M : 45 S = 5.8 L : 41.9 M : 52.3 S

When Mids of various constitutions are crossed with Shorts known not to carry factors conditioning Mid, the results again fall into two groups, as shown in Table VII. Three types of Mids give ratios approaching 2 Short : 1 Mid : 1 Long. The experimental data were 243 Shorts : 125 Mids : 126 Longs, or 49.2 Shorts : 25.5 Mids : 25.3 Longs. A Mid of constitution $a\,M_a - m_b \cdot a\,m_a - M_b$, on the other hand, should give a ratio of 50.0 Shorts : 47.5 Mids : 2.5 Longs when crossed to a Short without the M genes. Unfortunately, only two such families have been grown, therefore the numbers available are small. But there is reasonable

Table VIII

Mids Crossed with Shorts which are known to carry Mid factors

$a\,M_a - m_b \cdot a\,m_a - m_b \times A\,M_a - m_b \cdot a\,m_a - m_b = 16.7\,L : 33.3\,M : 50.0\,S$
$a\,M_a - M_b \cdot a\,m_a - m_b \times A\,M_a - m_b \cdot a\,m_a - m_b = 15.0\,L : 35.0\,M : 50.0\,S$
$a\,M_a - M_b \cdot a\,m_a - m_b \times A\,M_a - M_b \cdot a\,m_a - m_b = 14.4\,L : 35.6\,M : 50.0\,S$

$a\,M_a - m_b \cdot a\,m_a - M_b \times A\,M_a - m_b \cdot a\,m_a - m_b = 1.7\,L : 48.3\,M : 50.0\,S$
$a\,M_a - m_b \cdot a\,m_a - M_b \times A\,M_a - M_b \cdot a\,m_a - m_b = 2.2\,L : 47.8\,M : 50.0\,S$
$a\,M_a - m_b \cdot a\,m_a - M_b \times A\,M_a - m_b \cdot a\,m_a - M_b = 0.25\,L : 49.75\,M : 50.0\,S$

Table IX

Results from crossing heterozygous Mids with Shorts known to carry Mid

No.	Cross	Origin Female	Origin Male	Form of Progeny		
				L	M	S
1206	M × S	A (wild plant)	(M(M × L) 32 × SC) 37	0	12	15
1208	M × S	A (wild plant)	(M (M × L) 32 × SC) 2	3	17	13
1212	M × S	A (wild plant)	(SA × MA) 2	3	24	21
1210[1]	M × S	A (wild plant)	(M (M × L) 32 × SE) 1	7	37	40
1308	M × S	(MD 18 × LA) 17	(SA × MA) 2	4	7	12
1321	M × S	(MD 18 × LA) 17	(M (M × L) 32 × SC) 37	3	8	21
1304	M × S	(MD 18 × LA) 12	(SA × MA 19) 2	2	46	66
1305	S × M	(SA × MA 19) 2	(MD 18 × LA) 12	2	41	68
1302	S × M	(SA × MD 18) 43	(MD 18 × LA) 17	20	54	71

---

1) Short not known to carry Mid.

agreement with expectation in the figures 45 Shorts : 36 Mids : 5 Longs, a ratio of 52.3 Shorts : 41.9 Mids : 5.8 Longs.

The expectation for Mids crossed with Shorts known to carry Mid are listed in Table VIII. All of the possible types are given; though naturally the number of crosses possible is larger, since any given type of Mid may be used as a Short (plus gene A) and *vice versa*. In one group of crosses the ratio approaches 50 Short : 35 Mid : 15 Long. In the other group the ratio approaches 50 Short : 48 Mid : 2 Long.

Table IX presents a series of results from such unions. The Shorts used are known to carry Mid factors, because of their behavior in other crosses, with one exception. It is rather difficult to say just where each of these families belongs on account of the small numbers involved. From the last three families, Nos. 1304, 1305, and 1302, where numbers are fairly adequate, however, one is forced to the conclusion that here again the two decidedly different ratios are found. There is hardly anything in common with the ratio 134 Shorts : 87 Mids : 4 Longs, and the ratio 71 Shorts : 54 Mids : 20 Longs.

Considering all of these data together, I feel that there is a fair proof of the genetic hypothesis submitted.

Überreicht vom Verfasse

Sonderabdruck
aus den
**Verhandlungen des V. Internationalen Kongresses
für Vererbungswissenschaft
Berlin 1927**

**Supplementband I der
Zeitschrift für induktive Abstammungs-
und Vererbungslehre 1928**

Verlag von Gebrüder Borntraeger in Leipzig

E. M. East:

Heredity in the Genus Fragaria with Special Reference to the False Hybrids of Millardet

Heredity in the Genus Fragaria with Special Reference to the False Hybrids of Millardet

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The work of Millardet (1894) on *Fragaria* is commonly cited in textbooks of Genetics as an example of inheritance governed by laws somewhat different from those which are ordinarily effective. Millardet himself was of this opinion. In the introduction to his paper, he says: — "Gärtner states that we know of no case where the characteristics of either of the two species composing a hybrid have been transmitted as a whole. All of the physiologists who have occupied themselves with hybridization are of the same opinion. My intention, in this work, is to demonstrate that in the genus *Fragaria* the offspring obtained from the hybridization of certain species form an exception to this rule. They reproduce completely the specific type of the father or of the mother, and resemble in consequence the one or the other without uniting in themselves any of the distinctive characters of the two interacting species."

The evidence upon which Millardet bases his conclusions is briefly as follows:

The first cross described was between *F. vesca* var. *alba* and an hermaphroditic variety of *F. chiloensis*. One of the four resulting hybrids resembled the mother in every respect except that the fruit was red. Millardet evidently considers this last point to be of no importance; but to the modern geneticist it is proof that the plant was a true hybrid and not the result of induced parthenogenesis or polyembryony. Since this individual exhibited only the characters of *F. vesca*, it probably was the result of adventitious pollination from a red-fruited plant of that species. The other three hybrids resembled the male parent, *F. chiloensis*, from which, the author says, "it was almost impossible to distinguish them." These plants were almost completely sterile, but did yield a few seeds when left unprotected against foreign pollen. The resulting plants were like *F. chiloensis*.

In another cross, *F. elatior* × *F. grandiflora* var. Globe, 15 plants were produced, of which 14 were like the female parent and 1 like the male parent.

In a third cross, *F. vesca* × *F. grandiflora* var. Globe, 1 plant resulted. It was like the male parent.

In a fourth cross, *F. vesca* × *F. grandiflora* var. Ananas, 24 plants were obtained. Of these, 23 were like the female parent and 1 like the male parent.

In the remaining crosses, the progeny resembled the female parent, which happened in all cases to be either *F. vesca* or *F. elatior*.

In 1907, Solms-Laubach reported some crosses between a pistillate form of *F. virginiana* and *F. elatior*, in which the results of Millardet were assumed to have been substantiated. He says: —

„Aus den Samen der *Fragaria virginiana* war also hier fast ganz reine *elatior* aufgegangen, es war ‚fécondation sans croisement' in schönster Form eingetreten."

We have then to interpret (1) cases in which supposed hybrids resembled the female parent and bred true, (2) cases in which actual hybrids resembled the female parent and bred true to these specific characters, (3) cases where the hybrids resembled the male parent, and (4) cases where these patroclinous hybrids bred more or less true to the characteristics which they exhibited in the first hybrid generation.

The evidence which I wish to present in brief has been obtained at the Bussey Institution of Harvard University during the past eight years. The cytological observations were made by Mr. K. Ichijma (1926); the pedigree culture experiments were largely carried out by Dr. A. J. Mangelsdorf.

We can come to no decision at this time regarding the alleged matroclinous plants. It has been generally assumed that induced parthenogenesis or induced apogamy — presumably polyembryony — is the basis for Millardet's observations of plants resembling the female parent. It is quite possible that this is the case. But it must be noted that neither the experiments of Millardet nor of Solms-Laubach were properly safeguarded against error. All of the records of plants resembling the female parent and breeding true to their characters may be instances of accidental pollination with pollen from plants of the same species. In the case of the cross between *F. vesca* var. *alba* and *F. chiloensis*, one may conclude unhesitatingly that *vesca* pollen is the effective cause. In the other cases, the matter is still open.

During our earlier experiments, with methods of guarding against contamination much more effective than those used by Millardet, we obtained numerous plants resembling the female parent, plants which were 2 × in chromosome number and which bred true. If our observations are correct, the phenomenon is one of induced parthenogenesis with a later doubling of the chromosome number. A cross was made between white-fruited and red-fruited varieties of *F. vesca*, — the difference here being that of a single factor with dominance of red fruit. Plants of the F_1 generation were pollinated with pollen from other species. Matroclinous plants resulted which were in part red-fruited and in part white-fruited, proving, — if the observations are valid — that the induced development is gametic rather than zygotic.

But we cannot be certain of our observations. The pollen of all of the *Fragaria* species is very small and very light. Precautions which serve in experiments with other plants do not serve here. And when we made our crosses under conditions which absolutely excluded errors due to contamination, no matroclinous plants were obtained. We are continuing these experiments, and expect shortly to be able to draw definite conclusions in this regard. Fortunately, the matter of matroclinous plants is the least interesting. The interpretation of the patroclinous hybrids is more important.

The *Fragaria* species fall into three groups based upon the gametic number of chromosomes. The 7-chromosome group includes the following hermaphroditic species which resemble one another closely: — *F. vesca* L., *F. mexicana* Schlecht., *F. americana alba* Porter, *F. bracteata* Heller, and *F. californica* Cham. & Schlecht. One species, *F. elatior* Ehr., which is usually hermaphroditic, has 21 chromosomes. The 28-chromosome group, which contains species that are sometimes dioecious, includes *F. virginiana* Duchesne, *F. glauca* Watson, *F. chiloensis* L., and all of the garden varieties thus far tested, commonly placed under the species *F. grandiflora* Ehr. There appears to be no wild species having 14 chromosomes; but in a cross between *F. bracteata* and *F. Helleri*, a *vesca* variety, a 14-chromosome plant appeared which bred true to its characteristics, including the chromosome number.

Crosses between species having the same chromosome number behave in a perfectly normal manner. Such differences as the parents exhibit segregate and recombine as expected. Moreover, our experience with crosses between *F. chiloensis* and *F. virginiana*, as well as the historical evidence, lead us to believe that Duchesne was correct in tracing the descent of the cultivated strawberry to these two species. Three pistillate plants of *F. chiloensis* were brought from Chile to France by M. A. Frezier. These clons were incapable of self-fertilization, of course, but bore well when grown together with either *F. elatior* or *F. virginiana*. Since the hybrids between *F. chiloensis* and *F. virginiana* are completely fertile, while hybrids between *F. chiloensis* and *F. elatior* are rather sterile, and since all garden varieties combine the characteristics of *virginiana* and *chiloensis*, we have reason to believe that no other species have been concerned in the origin of the modern strawberry.

The results obtained when crosses were attempted between species carrying different chromosome numbers may be described briefly as follows: —

7-chromosome species × *21-chromosome species*, — *F. vesca* crossed with *F. elatior* seeded easily; but of 600 seeds, only 4 germinated. All of the seedlings died within two weeks. The reciprocal cross was unsuccessful.

7-chromosome species × *28-chromosome species*, — flowers of *F. vesca* pollinated from *F. chiloensis* nearly always produce fruit; but only 6 seedlings have been obtained from over 1200 seeds planted. Four of these plants were dwarfs which persisted for many months, developing many tiny leaves, but gaining very little in bulk. The other two hybrids were vigorous plants which attained the size of the parental plants in about 6 months. *These plants* resemble the male parent, *F. chiloensis*, very closely indeed in shape of leaf, texture of leaf, hairiness, and in the production of runners. Only one has blossomed, the flowers being almost identical with the male parent.

Several crosses have been attempted between *F. americana* and *F. glauca*. 66 seeds were obtained, but only 1 dwarf plant resulted.

F. vesca, on the other hand, crosses readily with the pollen of *F. glauca*. Five plants have been obtained from about 250 seeds. Four were dwarfs. The fifth plant is more vigorous but less than half the size of either of the parental species. It resembles the *glauca* parent in its vegetation. The flowers, though numerous, are abnormal, with greenish petals and rudimentary anthers.

F. vesca rosea crosses with *F. glauca* somewhat less readily. 76 seeds have yielded 7 plants, 5 being miniature dwarfs, 1 a dwarf of somewhat larger size, and 1 a flowering plant about one-third the size of plants of the parental species. This last plant hardly resembles either parent, but is more nearly like *glauca* than *vesca*.

F. vesca sets fruit rather freely with pollen from *F. virginiana*. From 500 seeds, 22 plants have been obtained. One is pure *vesca*. Eleven are miniature dwarfs. The other 10 plants are vigorous and flower freely; but the flowers are small and are completely sterile in back crosses. Each of these plants resembles the *virginiana* parent very closely, particularly as to shape and texture of leaves.

F. bracteata with pollen from *F. virginiana* yields but few seeds, though these seeds germinate fairly well. From 24 seeds, 12 plants were obtained. There were 2 plants apparently identical with the *bracteata* parent except that they had pistillate instead of hermaphroditic flowers. The other 10 plants were normal hybrids resembling the 28-chromosome parent except in details of leaf-shape. The plants produce normal flowers and a small amount of pollen; but selfing and back-crossing have not been successful.

In crosses between 7-chromosome species and 28-chromosome species, therefore, three types of plants have been obtained. There are first individuals resembling the mother which are completely fertile. These plants evidently are either the result of induced parthenogenesis, of induced apogamy, or of accidental selfing. There are two types of true hybrids, one is a dwarf, the other resembles the 28-chromosome male parent.

No successful crosses have been made between 28-chromosome species pollinated with pollen from 7-chromosome species.

28-chromosome species × 21-chromosome species, — a cross between *F. virginiana* and *F. elatior* has given a 90 per cent seed germination, much the best of any of these hybrids. The resulting plants exhibited a high degree of variation in vigor, yet the dominance of *F. elatior* is very striking. Many of the F_1 plants can scarcely be distinguished from the pure species except by their sterility. The reciprocal cross has never been successful.

It will therefore be seen that we have corroborated three of Millardet's observations of patrocliny, — those in which 7-chromosome species are crossed with 28-chromosome species. The plants are true hybrids, and the patrocliny is merely the dominant influence of the higher number of chromosomes. The fourth case of Millardet, *F. elatior* (21) × *F. grandiflora* (28) we have never been able to repeat. The case cited by Solms-Laubach, *F. virginiana* (28) × *F. elatior* (21), accords exactly with our experience. Evidently in this instance the higher number of chromosomes of the female parent *F. virginiana* is unable to dominate the characters exhibited by *F. elatior*. This phenomenon is simply a statement of fact, however, there being no question but that true hybrids have been obtained. Millardet's observation of patroclinous hybrids breeding true has not been corroborated. Such hybrids when selfed have yielded no progeny. But it is not improbable that he was correct in this regard, in the sense that patroclinous hybrids may sometimes yield offspring when fertilized with the pollen of the pure species which they resemble.

There is only one other point to be mentioned, the frequent occurrence of dwarf hybrids. We have suspected that perhaps these are haploid plants. Unfortunately, they present difficulties to the cytologist since they do not flower and their root system is meagre. Mr. S. H. Yarnell of my laboratory has been able to make root-tip counts in only two instances. These counts did not show haploidy.

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The Genetics of the Genus Nicotiana

Overdruk uit:

BIBLIOGRAPHIA GENETICA IV, 1928

’s-Gravenhage, Martinus Nijhoff

THE GENETICS OF THE GENUS NICOTIANA

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CONTENTS

Page
INTRODUCTION 243
CHROMOSOME NUMBERS IN NICOTIANA. . . . 249
INTER-SPECIFIC HYBRIDS IN NICOTIANA. . . 253
Pre-Mendelian investigations, 253; *Post-Mendelian investigations*, 256; *Cytological behavior of* F_1 *inter-specific hybrids and certain back crosses*, 267; *Origin of new polyploid forms through hybridization*, 276; *The behavior of partially fertile hybrids*, 279; *The behavior of fertile hybrids*, 285.
a. Inheritance of certain morphological characters in the alata-group, 286;
b. Inheritance of self-sterility in the alata-group, 288.
INTRA-SPECIFIC HYBRIDS IN NICOTIANA . . 291
Nicotiana Tabacum, 291; *Nicotiana rustica*, 301; *Nicotiana longiflora*, 302; *Nicotiana acuminata*, 306.
RELATIONSHIPS OF NICOTIANA SPECIES . . 307
BIBLIOGRAPHY 308

INTRODUCTION

The genus *Nicotiana* was highly favored as material by the older hybridizers (KÖLREUTER, 1761—1765; SAGERET, 1826; GÄRTNER, 1849; GODRON, 1863; NAUDIN, 1865; FOCKE, 1881). This predilection was not unreasonable. The plants of the various species grow well on all ordinary soils; the flowers are easy to castrate and to cross; and numerous seeds are produced, which retain their viability over considerable periods. Few genera have so many natural advantages as genetic material. Nevertheless, there are serious difficulties in the way of a thorough-going genetic analysis of the group. The most recent generic revision (COMES 1899) lists 41 species. Since this date, about 30 additional species have been described. Yet it appears to be impossible to bring together living specimens of more than one-third this number; and even some of these species are difficult to determine. Moreover, if my information is correct, no single one of the great herbaria of the world contains specimens of over one-half of the described forms. There are two reasons for this condition: (*a*) many of the original descriptions are indefinite and contain no figures, making identification difficult; (*b*) several species have been collected but once.

Nicotiana is essentially a new world genus, the center of distribution being Central America and northwestern South America. One species, *N. suaveolens* LEHM. is found in Australia and possibly in various islands of the south Pacific. Three other species have been listed from the region of the Malay Peninsula; but it is not certain that they are not merely varieties of *N. suaveolens* LEHM., of which COMES describes ten. The variety being grown in American botanic gardens and genetical institutes to-day is apparently *vincaeflora* COMES.

Nicotiana was divided into four sections by G. DON (1838); viz., I, TABACUM; II. RUSTICA; III. PETUNIOIDES; and IV. POLIDICLIA. This classification has been accepted by DUNAL (1852) and COMES (1899). EAST (1912) and SETCHELL (1912) both agree, however, that *N. quadrivalvis* PURSH and its variety *multivalvis* GRAY, which are the lone representatives of Polidiclia, are merely varieties of *N. Bigelovii* WATSON. They differ essentially from *N. Bigelovii* WATSON only in the number of locules in the capsule. The chromosome numbers are the same. They cross easily with *N. Bigelovii* and give fertile progeny; and all three forms overlap in distribution.

If the section *Polidiclia* is abandoned, the species upon which some genetic work has been accomplished may be classified as follows. COMES (1899) is followed roughly. The reader is also referred to MILLÁN (1926).

Section I. Tabacum Don.

N. Tabacum L. Numerous varieties exist. The distinctive characteristics of these types are variations in flower color (various shades of red and white), flower size and shape, teratological mutations in pistil, stamens, corolla, and calyx; shape and texture of leaves, and habit of growth (whether definite or indefinite). Various authors have tried to refer existing varieties to from four to six typical forms; but as all varieties cross freely, little is to be gained by such efforts.

Syn. Kölreuter (1761—5), *vulgaris, perennis, Transylvanica, albiflora, latissima*; Gärtner (1849), *macrophylla, Marylandia, magnifolia, petiolata, Chinensis, plantaginea, acuminata, Lehmanni, latissima, angustifolia, lanceolata, grandiflora*; Godron (1863), *auriculata.*

N. Rusbyi Britton. Its characteristics are intermediate between *N. Tabacum* and *N. tomentosa.* The flowers of this species have a slight tendency to zygomorphism. The stamens protrude beyond the corolla.

N. tomentosa R. et P. Genetic behavior shows that this species is a true *Nicotiana,* though Comes has referred it to *Lehmannia.* It shows a close relationship to the two preceding species on the one hand, and to *N. glutinosa* on the other hand. (Cf. Setchell 1912).

Section II. Rustica Don.

a. Foliis cordatis v. subcordatis ovatis Comes.

N. rustica L. Numerous interfertile varieties of this species exist, characterized by yellow-green, hypocrateromorphous flowers.

Syn. Gärtner (1849), *pumila*; Malloch & Malloch (1924), *persica.*

N. glutinosa L. This species, with its yellow-red, pronouncedly zygomorphic flowers, shows relationship both with the section Tabacum and the section Rustica.

N. paniculata L. This form is well known. A large-flowered variety is common; viz. *cerinthoides* (Horn) Comes.

N. solanifolia Walp. According to description, this plant differs from the next species by having a longer and woolier corolla (5 times calyx in length), and a 4-valved capsule. The form used by Christoff (1928) was collected in Chile. It was a paniculata-like type with larger and yellower flowers, and thus corresponded fairly well with the Prodromus description; but the pubescence was not wooly and the capsules were 2-valved.

N. cordifolia Phil. This species may be a variety of the preceding one. It is supposed to have flowers 3 times the length of the calyx with short instead of long hairs. I have not seen the forms used by GOODSPEED and CLAUSEN under these two names; but thus far there seems to be no very good reason for separating the forms listed as *N. solanifolia* and as *N. cordifolia* from *N. paniculata.*

N. glauca GRAH. No confusion exists regarding this wellknown arboreal species.

b. *Foliis ovatis vel ovato oblongis* COMES.

N. Langsdorffii WEINM. This species is the connecting link between the Rustica and the Petunioides sections, and really belongs to the latter (LOCK 1909). It gives fertile hybrids both with *N. alata* and with *N. Sanderae.*

N. undulata R. et P. I received this species from the experimental station at Scafati, Italy, in 1909. These plants were apparently correctly determined; but various species from the Petunioides section are being grown under the name.

Section III. Petunioides DON.

A. Longiflorae COMES.

a. Grandiflorae COMES.

N. sylvestris SPEG. et COMES. Morphologically this species in the connecting link between the Tabacum and the Petunioides sections. Genetically, it might well be considered to be the most specialized of the Tabacum group.

N. alata LK. et OTTO. The plant in genetic use is var. *grandiflora* COMES (*N. affinis* MOORE). It is assumed to be a large-flowered variety of *N. alata persica* (LINDLEY) COMES, described by LINDLEY as *N. persica* in 1833. The plant described by LINK and OTTO, and used by COMES as the type, may very well be a hybrid between one of the larger flowered varieties and *N. Langsdorffii* WEINM. Similar plants with medium sized flowers either greenish yellow or white, are obtained from this cross.

Syn. GÄRTNER (1849), *Persica, commutata* (possibly an *alata-Langsdorffii* hybrid).

N. Forgetiana HORT. SAND. This species is not supposed to be in cultivation. It was used by SANDER and SONS in crosses with *N. alata* to produce the hybrids known to horticulturists as *N. Sanderae.* The firm claims never to have sent out seed from the pure species, which may be correct; but I obtained seed from a GRAY Herbarium sheet coming from a California botanist, which produced plants agreeing with SANDER's plate in the Botanical Magazine (8006). These plants bred true to the small flowers and red color characteristic of *N. Forgetiana,* and I have used the specific name in at least one publication (EAST 1913); but they may have been segregates from *N. Sanderae.*

N. noctiflora HOOK(?) (Bot. Mag. tab. 2785). This species and *N. acuminata* appear to be alike except that the former has some reddish pigment on the exterior of the corolla lobes. Type specimens were collected of both forms by PHILIPPI at Santiago, Chile. Plants from seeds labeled *N. noctiflora* var. *albiflora,* sent by COMES to SETCHELL and by him (1912) referred doubtfully to *N. noctiflora,* are a small variety of *N. longiflora* CAV. (possibly *N. plumbaginifolia* VIV.). I have had similar plants from the same source. It is questionable whether any modern geneticist has had true *N. noctiflora,* if indeed such a species exists distinct from *N. acuminata.*

N. acuminata GRAH. (Bot. Mag. tab. 2919). GOODSPEED (1912) has used three varieties of this plant: (*a*) *N. acuminata grandiflora* COMES from the Cambridge Botanical Garden, (*b*) a smaller-flowered type from La Mortola identified as *N. acuminata parviflora* COMES, and (*c*) a still smaller-flowered plant which was thought to have come from Fort Bidwell, California.

N. caudigera PHIL. This form is very much like *N. acuminata,* having long-petioled leaves, cordate below and lanceolate above. The flowers differ from *N. acuminata* in having the corolla tube constricted at the base, but swelling just above the calyx to a diameter that continues the same to the throat. Under greenhouse conditions at Boston, Massachusetts, U.S.A., the plants die soon after flowering.

N. suaveolens LEHM. Though COMES lists ten varieties, the only plants used in genetical work appear to belong to variety *vincaeflora* COMES. They correspond closely with Bot. Mag. tab. 673, which is there listed as *N. undulata,* New Holland tobacco.

N. attenuata TORR. The distinguishing feature of this species is that the leaves are attenuated to very long petioles.

Syn. CHRISTOFF (1928), *viscosa*(?).

N. Bigelovii WATS. This evil-odored *Nicotiana* is very common; but I have never seen the smaller-flowered variety *Wallaceii* GRAY. There is considerable question as to whether the two types are more than extremes of a linear series.

Syn. *N. quadrivalvis* Pursh. and *N. quadrivalvis* var. *multivalvis* Gray. (See *N. Clevelandi* Gray).

b) *Gracilif lorae* Comes.

N. longiflora Cav. This species is well known in the gardens of Europe. There are several varieties differing mainly in size of flower.

N. plumbaginifolia Viv. The plants coming from European gardens are all small varieties of *N. longiflora* Cav. I have not been able to see the plate accompanying the original description (En. Pl. hort. J. C. Dinegro); but there is nothing in the description itself to indicate that it is distinct from *N. longiflora* Cav. *N. plumbaginifolia* is said to be an annual, with insertion of the stamens near the throat of the corolla; while *N. longiflora* is said to be a perennial, with insertion of the stamens further down the corolla tube. These characteristics are indefinite. All Nicotianas appear to be perennial under optimum conditions. And the insertion of the stamens in the two forms is similar if one takes into consideration the difference in corolla length. I am inclined to believe that all of the plants now grown under the name *N. plumbaginifolia* are *N. longiflora.* The plant figured by Setchell as *N. noctiflora* Hook(?) is of this type.

Clausen (1928) places a form which he calls *N. angustifolia* in this group. He gives no authority for the name.

N. repandi Willd. An excellent illustration of this plant is given by Setchell (1912, plate 27).

B. Breviflorae Comes.

a. *Americae borealis.*

N. nudicaulis G. Watson. This species differs from the following by its long, sparsely flowered central spike.

N. trigonophylla Dun. This is the common species of southwestern North America.

N. Palmeri Gray. This species differs from the preceding in its somewhat larger flowers. The two types probably do not deserve specific distinction.

N. Clevelandi Gray. This species appears to be a small-flowered long-leaved relative of *N. Bigelovii.* It is placed in the *N. Bigelovii* group by Clausen (1928).

b. *Americae australis.*

N. angustifolia R. et P. This species is one of the *acuminata* group

I have received var. *crispa* Comes (*N. Cavanillesii* Dun.) from Scafati. It is marked by glaucous leaves and stems.

In addition to these species Clausen has reported the chromosome number (24) in a species termed *N. Clarionensis*. He gives no authority for the name, and I have been unable to find it in the literature.

CHROMOSOME NUMBERS IN THE GENUS NICOTIANA

The chromosome numbers have been determined for the following species of *Nicotiana*. In most cases the count was made at meiosis, although Christoff (1928) checked nearly all of his determinations on root tip material. In two cases, *N. tomentosa* and *N. plumbaginifolia* (= *N. longiflora*), Christoff made counts only upon root tips.

$N = 8$ or 9

N. Langsdorffii Weinm.	Goodspeed $n = 9$ (1923, 1924); Christoff $n = 8$ (1928)
N. alata Lk. et Otto	Goodspeed $n = 8$, 9 or 10 (1923); Goodspeed $n = 9$ (1924); Christoff $n = 8$ (1925, 1928); Ruttle $n = 9$ (1927)
N. Sanderae Hort. Sand.	Christoff $n = 8$ (1928)

$N = 10$

N. longiflora Cav.	Goodspeed (1924); Christoff (1928)
" " var. (= *N. plumbaginifolia* Viv.)	Christoff (1928)
(= *N. angustifolia*?)	Clausen (1928)

$N = 12$

N. Rusbyi Britt.	Brieger (1927)
N. tomentosa R. et P.	Christoff (1928)
N. glutinosa L.	Goodspeed (1923, 1924); Christoff (1928)
N. paniculata L.	Goodspeed (1923, 1924); Christoff (1928)
N. caudigera Phil.	Christoff (1928)

N. glauca Grah.	Goodspeed (1923, 1924); Christoff (1928)
N. attenuata Torr.	Clausen (1928); N = 24, Christoff (1928)
N. repanda W.	Clausen (1928)
N. sylvestris Speg. et Comes	Goodspeed (1923, 1924); Christoff (1928)
N. acuminata Grah.	Goodspeed (1923, 1924); Christoff (1928)
N. trigonophylla Dun.	Christoff (1928); Clausen (1928)
N. Palmeri Gray	Christoff (1928)
N. solanifolia Walp.	Christoff (1928); Clausen (1928)
N. cordifolia Phil.	Clausen (1928)
	N = 16
N. suaveolens Lehm.	Goodspeed n = 18 (1923); Christoff n = 16 (1928) Admitted to be 16 by Goodspeed and Clausen (1917) after seeing Christoff's preparations.
	N = 24
N. Tabacum L.	White (1913) 3 vars.; Palm (1923); Goodspeed (1923, 1924), 5 vars.; Rybin (1927); Christoff (1928), 4 vars.
N. rustica L.	East (1921); Goodspeed (1923, 1924), 3 vars.; Rybin (1927); Christoff (1928)
N. Bigelovii Wats.	Goodspeed (1923; 1924); Christoff (1928)
„ „ var. (= *N. quadrivalvis* Pursh.)	Christoff (1928)
N. Bigelovii var. (= *N. multivalvis* Lindl.)	Christoff (1928)
N. Clevelandii Gray (var. of *N. Bigelovii*?)	Clausen (1928)
N. nudicaulis	Goodspeed (1923, 1924); Christoff (1928)

N. attenuata Torr.	Christoff (1928); N = 12, Clausen (1928)
,, ,, var. (recd. under name *N. viscosa* Lehm.)	Christoff (1928)
N. Clarionensis?	Clausen (1928)

Apparently there are no special peculiarities in the chromosome behavior of the various *Nicotiana* species. The behavior of each species is regular, with the possible exception of the *Langsdorffi-alata-Sanderae* group. Some irregularities have also been found in teratological varieties; viz., a fasciated *N. Tabacum* (White 1913) and a petaloid *N. Tabacum* (Goodspeed 1924). Among the various species, there are fairly well defined differences in chromosome size and shape at meiosis under similar conditions of fixation and staining, and great differences even in the same species at meiotic and at mitotic divisions.

No particular difference of opinion exists regarding the number of chromosomes possessed by the various *Nicotiana* species, except in two instances. The first case is the group of species consisting of *N. Langsdorffii*, *N. alata*, and *N. Sanderae*, — which behave similarly. Goodspeed (1923, 1924) obtained counts of 8, 9, and 10 in *N. alata*, and suggested that non-disjunction accounts for the variation from the number 9. Later, Christoff (1925) counted and figured 8 as the haploid number. In 1927, Miss Ruttle published an excellent paper on the cytology of *N. alata*. She studied embryo sac mother cells, pollen mother cells, and root tips in several fixatives, including Belling smears. From root-tip cells, she was able to get about twenty-five counts giving 18 chromosomes. There were 4 long and more or less V-shaped chromosomes, 2 chromosomes with satellites, and 12 smaller chromosomes.

Numerous counts were also made of pollen mother cells and embryo sac mother cells on a considerable number of plants. The largest number of counts was made at the second metaphase of the pollen mother cells. Single counts were divided into 16 cases of 8, 397 cases of 9, and 25 cases of 10. Double counts gave 8 cases of 8 and 10, and 89 cases of 9 and 9. Counts of other phases were similar. In view of these facts, plus the observation that the 8—10 distribution increases from 3 per cent in vigorous plants to 20 per cent in old plants, and in addition the observation that tetrad formation is often irregular, Miss

RUTTLE maintains that 9 is the correct haploid number for *N. alata.*

CHRISTOFF (1928), while not dogmatic, is inclined to think that 8 is the correct number for this group of species. He makes the following comment on Miss RUTTLE's work:

"It is possible that Miss RUTTLE's view is correct. But I must call attention to two points which should be weighed carefully before a decision is made. First, Miss RUTTLE figures 3 pairs of V-shaped chromosomes in her Figure 1*a* plus 2 pairs, the ends of which lie very close together (upper center). This latter configuration I believe to be a single pair of chromosomes. Second, and this is my critical point, in certain hybrids with species having 10 chromosomes as the haploid number, the reduction division of the pollen mother cells shows the number of univalents properly to be expected if the chromosome number for *N. alata* is x = 8. For example, in heterotypic spindles of *N. longiflora* × *Sanderae, N. longiflora* × *alata, N. plumbaginifolia* × *alata, N. plumbaginifolia* × *Langsdorffii, up to two lagging univalent chromosomes* occur and are often seen approaching the poles, whereas the bivalents are still at the equator (see figure 37); and *regularly more than two lagging chromosomes* appear during the homeotypic division."

Whatever the condition in *N. alata,* it presumably is the same in *N. Langsdorffii* and *N. Sanderae,* as shown by the behavior of their hybrids. Cytological preparations are also identical. I have seen CHRISTOFF's slides (his work being done in my laboratory), and his last argument on the basis of crosses with unquestioned 10-chromosome species deserves consideration. On the other hand, I am not convinced that 9 is not the correct number. Recently, Dr. KOSTOFF has made a number of slides of *N. Langsdorffii* (P. M. C. and root tips) in my laboratory, and there the counts were 9 and 18. Possibly 8-chromosome plants exist; but 9-chromosome plants seem to be the rule.

The second case is *N. attenuata* which CHRISTOFF determines as N = 24; and which CLAUSEN determines as N = 12. It is possible that *N. viscosa* LEHM. is the true determination of CHRISTOFF's plants. They came from a GRAY Herbarium sheet; but unfortunately the collector's data were misplaced and it is unknown whether the sheet came from North America or from South America. The characteristics of the plants, however, were practically identical with North American plants raised from seed obtained from GOODSPEED.

INTERSPECIFIC HYBRIDS IN NICOTIANA

Pre-Mendelian investigations

I have examined the work of KÖLREUTER (1761—1765), GÄRTNER (1849) and FOCKE (1881), and after reducing their specific names to those used in this paper, have listed the primary hybrids in Table 1. I think there is no question as to the proper species except in the case of *N. californica* NAUD. FOCKE (p. 286) says "this appears to be *N. attenuata* TORR. I have so listed it, but cannot be certain of the identification."

Table 1, as it stands, shows all of the primary hybrids tried. Those in which there was no stimulation of the ovary are marked x; parthenocarpy is marked p; weak embryo production, where the seeds obtained would not germinate, is marked w; hybrids are designated H. Naturally, the table is not all that one might desire to base conclusions upon. The most successful results are listed. But since all combinations were not tried an equal number of times, under similar conditions, it is possible (as is indeed shown by later investigations) that the true compatibility of the species is not shown. Nevertheless, I think that the results do show the trend of relationship in a general way. (See table I, page 254).

The following combinations, tested reciprocally, were successful both ways.

alata (9) — *Langsdorffii* (9). Intermed. Fertile.
suaveolens (16) — *glutinosa* (12). Intermed. Sterile. G. × s. difficult.
Tabacum (24) — *glutinosa* (12). Intermed. Sterile. Vigorous.
paniculata (12) — *suaveolens* (16). Like *suaveolens*. Sterile. S. x p. difficult.
Bigelovii (24)—*glutinosa* (12). Sterile. [GÄRTNER contradictory, B. × g.(?)].
rustica (24) — *paniculata* (12). More like *rustica*. Partially fertile.
suaveolens (16) — *Bigelovii* (24). More like *Bigelovii*. Sterile.

Generally speaking, the hybrid resembles the parent with the greater chromosome number. There seems to be no clear relationship between chromosome number and the difficulty of making the cross, though a difference in the ease or difficulty of making reciprocals is fairly well established.

TABLE I. — NICOTIANA HYBRIDS MADE BY THE EARLIER HYBRIDIZERS KÖLREUTER, GÄRTNER, GODRON, SAGERET, NAUDIN, FOCKE.

Female	Male	9 *N. alata*	9 *N. Langsdorffii*	12 *N. glutinosa*	12 *N. paniculata*	12 *N. glauca*	12 *N. acuminata*	16 *N. suaveolens*	24 *N. Tabacum*	24 *N. rustica*	24 *N. Bigelovii*	24 *N, attenuata?*
N. alata	9		H									
N. Langsdorffii	9	H		x	x	x		x	x	x	x	
N. glutinosa	12		x		w		p	H	H	x	H	
N. paniculata	12		H	H		x		H	H	H	H	
N. glauca	12		H		x				H			
N. acuminata	12		x	p	x			p	p	x	p	
N. suaveolens	16		H	H	H		p		H	x	H	
N. Tabacum	24	H	p	H	w		p	x		p	x	
N. rustica	24		w	w	H			H	H		H	H
N. Bigelovii	24		p	H	w			H	H	w		
N. attenuata?	24											

Legend: x, no stimulation; p, parthenocarpy; w, weak embryos; H, hybrids.

The following combinations were tested reciprocally and were unsuccessfull both ways.

glutinosa (12) — *Langsdorffii* (9)
acuminata (12) — *glutinosa* (12)
glauca (12) — *paniculata* (12)
Tabacum (24) — *Langsdorffii* (9)
rustica (24) — *Langsdorffii* (9)
Bigelovii (24) — *Langsdorffii* (9)
rustica (24) — *glutinosa* (12)
suaveolens (16) — *acuminata* (12)
Tabacum (24) — *acuminata* (12)

The outstanding feature of this list is the relative incompatibility of *N. Langsdorffii* with other species.

The following combinations were successful when tested as listed (the female parent first), but were unsuccessful in the reciprocal combinations.

paniculata (12) — *Langsdorffii* (9). Vigorous. Sterile. More like *Langsdorffii.*
glauca (12) — *Langsdorffii* (9). Weak. Sterile. More like *glauca.*
paniculata (12) — *glutinosa* (12). Weak. Sterile. Intermediate.
suaveolens (16) — *Langsdorffii* (9). Mod. vigorous. Sterile. More like *suaveolens.*
paniculata (12) — *Tabacum* (24). Weak. Sterile. More like *Tabacum.*
paniculata (12) — *Bigelovii* (24). Weak. Sterile. More like *Bigelovii.*
suaveolens (16) — *Tabacum* (24). Mod. vigorous. Sterile. Intermediate
rustica (24) — *suaveolens* (16). Weak. Sterile. Intermediate.
Bigelovii (24) — *Tabacum* (24). Vigorous. Sterile.
rustica (24) — *Bigelovii* (24). Vigorous. Sterile. More like *Bigelovii.*

One can hardly avoid the conclusion, particularly if one includes the evidence from crosses like *suaveolens—Langsdorffii* and *paniculata—Langsdorffii,* that the hybrid tends to resemble the parent with the greater chromosome number. It is not established, however, that the use of a species with a high chromosome number as the mother plant tends to make that cross easier than the reciprocal.

Perhaps the most interesting results of the pre-Mendelian hybridizers are those obtained from complex crosses. They made a considerable number of attempts to back-cross hybrids with their parents and to cross in other species; but all such attempts failed, except those involving *rustica—paniculata* and *rustica—Tabacum. Rustica* (24) — *Tabacum* (24) gave hybrids reciprocally with *paniculata* (12). *Rustica* (24) also yielded hybrids with pollen of *paniculata* (12)—*Tabacum* (24). Moreover, *rustica* (24)—*paniculata* (12), (as well as *rustica—paniculata—paniculata*) gave hybrids with both parents, with *rustica* (24)—*paniculata* (12)—*Tabacum* (24), with *Langsdor fii* (9), with *Tabacum* (24), with *glutinosa* (12), and with *paniculata* (12) — *Tabacum* (24). Thus, without counting reciprocals or other combinations involving the same species, there are three crosses involving three distinct species, *rustica* (24) — *Tabacum* (24) — *paniculata* (12), *rustica* (24) — *paniculata* (12) — *Langsdorffii* (9), and *rustica* (24) — *paniculata* (12) — *glutinosa* (12). And one of these crosses involves three different chromosome numbers. Surely one may expect some chromosome homology among species which exhibit such compatibility.

Post-Mendelian investigations

The post-Mendelian *Nicotiana* hybrids have been listed here with the hope that the data may serve as a guide toward a better understanding of the genetic relationships of the species; but conclusions must be drawn with caution, for two reasons. First, the various combinations possible have not been given an equal opportunity. CHRISTOFF's work (1928) is the only extended series of crosses where care was taken to give approximately equal opportunity to the crosses attempted. He selected fifteen species, and with a few exceptions, made all of the various combinations five times (in some cases, more). But the largest series of crosses (EAST 1912 and unpublished) cannot be compared with each other without some possibility of error. All of the attempts at crossing were made at least three times, yet since as high as one hundred attempts were made to get certain combinations a failure in one case and a success in another case is not conclusive evidence of a difference in compatibility. Second, the complete failures are designated as *x*, apparently parthenocarpic seeds as *p*, seeds with embryos and seeds which produced weak seedlings as *s*; but it is apparent that without cytological examinations the difference between *p* and *s* cannot be guaranteed.

In addition, attention is to be directed to two points. The vigor of the hybrids is rated by comparison with the average of the parents taken as 100 per cent. The germination of the seedlings is listed as normal (= 95—100 per cent) and in tenths of normal. EAST (1912) was criticized for marking the germination of certain *Nicotiana* hybrids as 100 per cent (Table V). This figure was meant only to indicate a normal percentage germination of over 95 per cent.

The *N. alata* group.

It is not certain that any geneticist has had true *N. Forgetiana*, as has been noted earlier; though possibly the plants used by LOCK (1909) and EAST (1913) were such. But since most of the plants now so common in the gardens of the world under the name *N. Sanderae* have 9 (8 according to CHRISTOFF 1928) chromosomes, and since the chromosome behavior is identical with that exhibited by *N. alata* and *N.*

Langsdorffii, we may take it that *N. Forgetiana* belongs to the same group. To be properly conservative, however, it is perhaps better to treat the work of Lock and of East as having been done with segregates of the horticultural species *N. Sanderae,* which was produced by crossing *N. Forgetiana* with *N. alata.*

Except on the basis of interfertility, these species are quite distinct

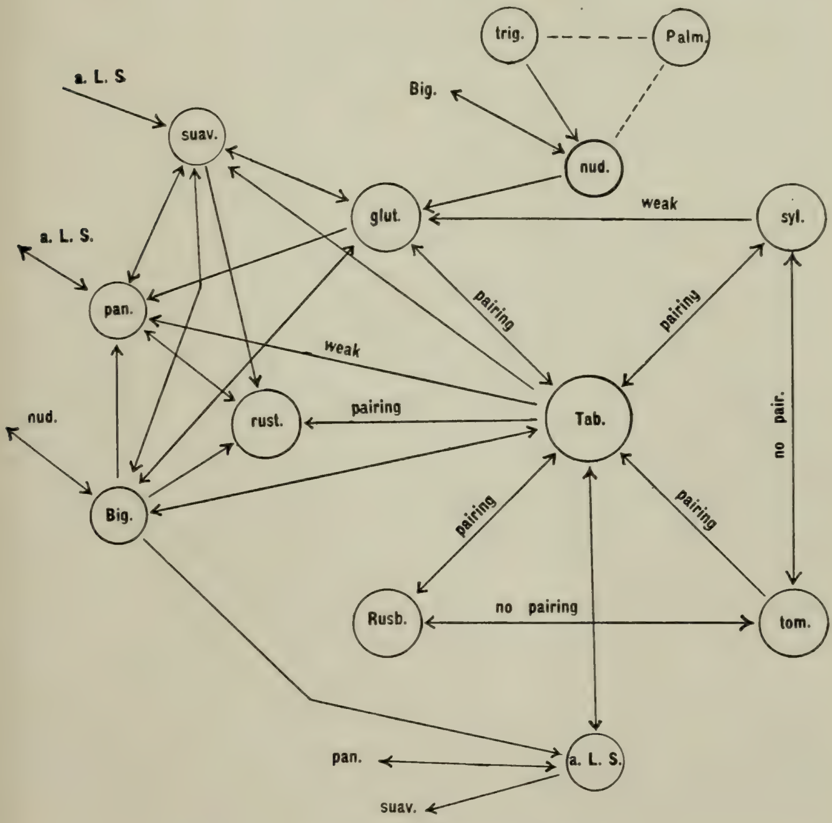

Fig. 1. Relations of Nicotiana species.

differing in a great number of characters. But when compatibility only is considered, they may well be taken as one group. *Alata, Langsdorffii,* and *Sanderae* all cross together in every possible combination, and their hybrids show a degree of fertility quite comparable with the pure species. Moreover, the compatibility of each member of the group with the less closely related species is sufficiently similar that one may assume that it would be identical under like conditions.

This is shown in Table II where I have compiled the different attempts at hybridization with the authorities for each.

With the 10-chromosome group, *N. longifolia* and *N. plumbaginifolia*, the members of the *alata* group hybridize with great difficulty. Christoff (1928) tried to cross both *N. alata* and *N. Langsdorffii* with pollen of *N. longiflora* and *N. plumbaginifolia*; but these attempts resulted in failure. East obtained *N. alata* × *N. longiflora*. The seeds germinated normally (90—100 per cent). The sterile plants exhibited 100 per cent vigor (a comparison with parental average), and an intermediate condition with regard to most characters. The reciprocal crosses are somewhat easier to make, the following being successful: *N. longiflora* × *N. alata* (Goodspeed and Clausen, East, Christoff *N. longiflora* × *N. Sanderae* (Malloch & Malloch), *N. plumbaginifolia* × *N. alata* (Lock, Christoff), *N. plumbaginifolia* × *N. Langsdorffii* (Christoff). These hybrids are all sterile, but show a vigor about equal to that of the parents. They are intermediate in character.

The group shows little compatibility with species having a higher chromosome number, though a fairly representative lot of attempts at crossing have been made. East succeeded in raising plants from three combinations. *N. alata* was fertile both ways with *N. paniculata*. And, strangely enough, both combinations showed a slight fertility. Seeds which did not germinate were produced by the F_1 of *N. alata* × *N. paniculata*. From the reciprocal F_1 generation *N. paniculata* × *N. alata*, three plants were raised to maturity. They were vigorous plants resembling *N. alata*, except in the flowers which were less decidedly lobed. Nothing is known about the cytological relationships. Nor is the source of the pollen known. The F_1 plants exhibited about 100 per cent vigor, and tended to resemble *N. alata* rather than *N. paniculata* The reciprocals were similar. The second combination was *N. alata* × *N. Tabacum*. The seedlings could not be brought to maturity. The third combination was *N. Langsdorffii* × *N. Bigelovii*. Seeds showed normal germination. The plants were 110 per cent in general vigor, sterile, and resembled *N. Bigelovii*.

The *alata* group appears to produce hybrids more freely when used as males than when used as females. Gärtner (1849) produced *N. glauca* × *N. Langsdorffii*, and says that it rather resembled *N. glauca*. Kostoff has crossed *N. glauca* with all three species, and has obtained backcrosses with *N. Langsdorffii*. The plants are very vigorous and, on the whole, should be called intermediate. I have already described *N. paniculata* × *alata*. GÄRTNER made *N. paniculata* × *N. Langsdorffii*, which he states to be very similar to *N. Langsdorffii*. LOCK (1909) also obtained weak and slow-growing hybrids from this combination, standing more closely to *N. paniculata*. They have also been obtained by EAST, GOODSPEED and CLAUSEN, CHRISTOFF, and KOSTOFF. I should take them to be intermediate in character. CHRISTOFF has also obtained the combination *N. paniculata* × *N. Sanderae*. Post-Mendelian workers have not been able to cross *N. suaveolens* with any of the *alata* group, but GÄRTNER was able to make the cross with the pollen of *N. Langsdorffii*. The hybrid was very similar to *N. suaveolens*. Apparently, no one has succeeded in raising hybrids of *N. rustica* made with pollen of *N. alata*, though EAST obtained seedlings; but EAST, CHRISTOFF, and KOSTOFF have obtained hybrids with pollen of *N. Langsdorffii*, and CHRISTOFF and KOSTOFF with pollen of *N. Sanderae*. The plants of *N. rustica* × *N. Langsdorffii* grown by

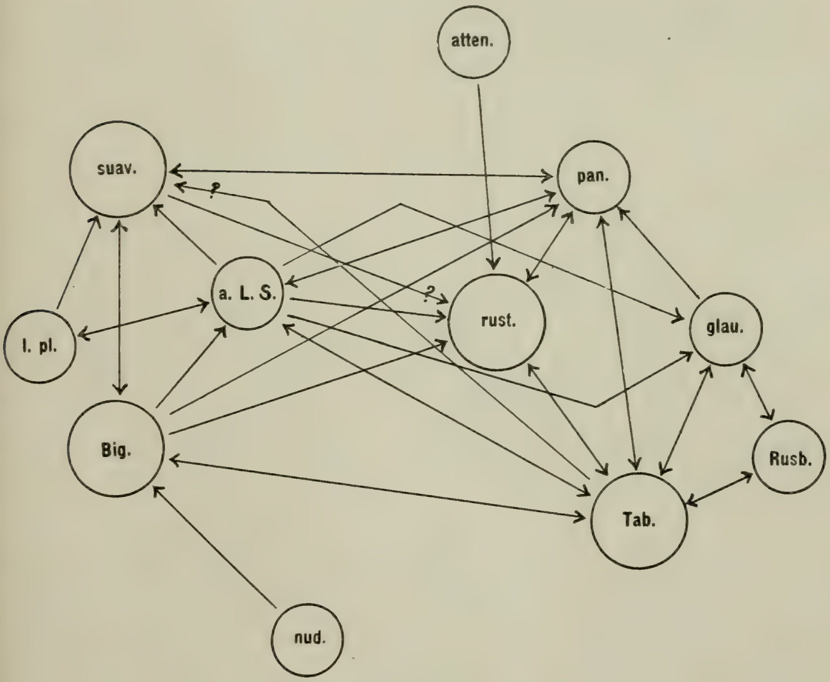

FIG. 2. Relationships of Nicotiana species.

East showed 110 per cent vigor. The germination of the seed was eight-tenths normal. Both the combinations, *N. rustica* × *N. Langsdorffii* and *N. rustica* × *N. Sanderae* obtained by Christoff and Kostoff, were quite weak. They were intermediate in character. Gärtner crossed *N. tabacum latissima* with *N. alata*. He records the growth as being very slow, but the plants finally reached a considerable height (50—100 cm.). The plants obtained by East and by Christoff were weak and slow-growing, showing 10 per cent in vigor. They resembled *N. Tabacum* rather than *N. alata*, except possibly in corolla shape. In leaf form the variety of *N. Tabacum* used was followed by the hybrid, though on a reduced scale. The hybrids *N. Tabacum* × *N. Sanderae* raised by Christoff and Kostoff were also weak, with great resemblance to *N. Tabacum*.

It should be noted here that, while in general characters these combinations of the *alata* group with other species were either intermediate or resembled the species with the higher chromosome number, certain color characters were always dominant. That is to say the blue pollen of *N. Langsdorffii* and the characteristic flower colors of *N. Sanderae* were exhibited in all of the F_1 hybrids, such as *N. Tabacum* × *N. alata* and *N. Tabacum* × *N. Sanderae*.

The *N. longiflora* group.

N. longiflora, and *N. plumbaginifolia* which, as I have said before, appears to be only a smaller-flowered variety of *N. longiflora*, cross with the *alata* group, — though with difficulty. When used as females with pollen from 9 other species, however, nothing but failure is to be recorded. Nor is this lack of compatibility to be accounted for by the great length of the pistils of these species, for reciprocal crosses also failed, except with *N. suaveolens* and *N. Tabacum*. East obtained the hybrid *N. suaveolens* × *N. longiflora* and Christoff was successful also when he used pollen from *N. plumbaginifolia*. The germination of the seeds was rather low (22% and 2%) in Christoff's experience with these two crosses; but the hybrids were quite vigorous. They resembled *N. suaveolens* except for the dominance of the bluish color on the outside of the corolla lobes of the paternal species.

N. acuminata.

N. acuminata shows little ability to hybridize with any of the *Nicotiana* species under consideration. It has been used repeatedly as female with 14 species, and as male with 13 species; but only one success is to be recorded. GOODSPEED and CLAUSEN produced *N. Tabacum* × *N. acuminata.*

N. glauca.

N. glauca has not been given a thorough testing with other species. No combinations have been made with *N. longiflora, N. plumbaginifolia, N. acuminata,* and *N. suaveolens.* Used as a female, it has given hybrids with the *alata* group (KOSTOFF), *N. Rusbyi* (KOSTOFF), *N. tomentosa* (KOSTOFF), and *N. Tabacum* (BRONGNIART, NAUDIN, GOODSPEED and CLAUSEN, KOSTOFF). The first three are vigorous, sterile hybrids, intermediate in nature. The last combination is very vigorous, sterile, and resembles *N. Tabacum.* KOSTOFF has made reciprocal crosses with *N. tomentosa* and *N. Tabacum.* They are similar. *N. Tabacum* × *N. glauca* is also recorded by NAUDIN, LOCK and EAST. GÄRTNER and KOSTOFF failed to obtain the cross *N. glauca* × *N. paniculata.* GÄRTNER's effort to obtain the reciprocal also failed, but EAST (also GOODSPEED and CLAUSEN) obtained plants 200 cm. high, intermediate in character, but becoming glaucous with age, and therefore seeming to stand closer to *N. glauca* at maturity. Other hybrids are *N. glutinosa* × *N. glauca* and *N. nudicaulis* × *N. glauca* obtained by GOODSPEED and CLAUSEN.

N. glutinosa.

N. glutinosa shows no compatibility with the *alata* or *longiflora* groups, or with *N. acuminata, N. trigonophylla* or *N. rustica.* In spite of being placed by taxonomists in the Rustica section of the genus, the closest affinity of this species is with *N. Tabacum,* with which it crosses reciprocally. The hybrid *N. glutinosa* × *N. Tabacum* was made many times, using different varieties of *N. Tabacum* as the pollen parent, by KÖLREUTER and by GÄRTNER. These authors agree that the plants are extraordinarily vigorous, some of them reaching nine feet in height. GÄRTNER states that *Tabacum* varieties *magnifolia, macrophylla, Marylandica* and *petiolata* gave identical forms when crossed on *glutinosa.* Despite some apparently contradictory statements, it appears that the hybrids stood closer to *Tabacum* than to *glutinosa.* With the latter conclusion CLAUSEN and EAST, who have made similar hybrids recently, will agree. The same type of sterile vigorous hybrids in the combination *N. Tabacum* × *N. glutinosa* has been made by KÖLREUTER, GÄRTNER, NAUDIN, EAST, CLAUSEN and KOSTOFF. If there is any difference exhibited in this combination over the reciprocal, I cannot discover it; but the earlier writers tend to emphasize the similarity to *Tabacum* where *Tabacum* is the mother-plant. *N. glutinosa* also crosses reciprocally with *N. Bigelovii* and with *N. suaveolens*. GÄRTNER certainly obtained the combination *N. glutinosa* × *N. Bigelovii quadrivalvis*, and probably the reverse, though he is contradictory on different pages (see FOCKE, p. 293).

GOODSPEED and CLAUSEN have made the combination using *Bigelovii* as the mother, and CHRISTOFF has made both combinations. The seeds germinate normally; the plants are vigorous, and resemble *N. Bigelovii* very closely. GÄRTNER's reciprocal hybrids of *N. glutinosa* and *N. suaveolens* were said to be intermediate. CHRISTOFF obtained a nearly normal germination of seeds from the combination *N. suaveolens* × *N. glutinosa*. The plants were vigorous, though sterile The habit of growth and the size and shape of the flowers were like those of *N. suaveolens*; but the leaves were petiolate and the flowers pink, like *N. glutinosa*. CHRISTOFF (also GOODSPEED and CLAUSEN) obtained the combination *N. glutinosa* × *N. sylvestris*, though he did not obtain the reciprocal cross. Though they flowered and appeared to be intermediate in their characteristics, the plants were very weak. CHRISTOFF (and GOODSPEED and CLAUSEN) also obtained weak sterile plants (one-tenth normal germination) from the cross *N. glutinosa* × *N. nudicaulis*. In habit, they were intermediate. The leaves were petiolate like *N. glutinosa*, and the flowers approached this species in color and shape, though about the size of those of *N. nudicaulis*. The reciprocal cross was not attempted. The hybrid *N. paniculata* × *N. glutinosa* was obtained by KÖLREUTER in a single example, by GOODSPEED and CLAUSEN, and by CHRISTOFF. Germination of the seeds was less than 1 per cent, but CHRISTOFF raised 5 hybrids. They were very weak when young, but quite vigorous later. They were distinctly intermediate in all characters, even to an intermixture of yellow and pink in the flowers. The reverse cross failed. GOODSPEED and CLAUSEN have produced the combination *N. glutinosa* × *N. glauca*.

N. paniculata.

N. paniculata used as a female forms hybrids with the *alata* group and with *N. glauca* and *N. glutinosa,* as already described. Used as a male it was fertile only with *N. alata* among the species that have been discussed.

N. paniculata is very closely related to *N. rustica,* as is shown by the partially fertile reciprocal crosses similar to *N. rustica* studied in such detail by KÖLREUTER and GÄRTNER and later by EAST. Their behavior will be described later.

Other combinations are not easy to make, and rarely succeed except when *N. paniculata* is used as the mother. GÄRTNER made reciprocal combinations of *N. paniculata* and *N. suaveolens* and obtained hybrids almost exactly like *N. suaveolens,* — if I am not in error following COMES in supposing that his *N. vincaeflora* LAG. is a variety of *N. suaveolens* LEHM. It is perhaps questionable, for modern attempts to make these crosses have failed. GÄRTNER apparently obtained the combination *N. paniculata* × *N. Bigelovii,* but not the reciprocal. The plants were similar to the pollen parent. EAST also obtained this cross and not the reciprocal. The hybrids showed normal vigor and resembled *N. Bigelovii* almost exclusively. KÖLREUTER obtained a single weak flowering plant, apparently resembling *N. Tabacum,* from the cross *N. paniculata* × *N. Tabacum.* He did not obtain the reciprocal combination. EAST obtained weak plants which died before flowering in both combinations.

N. Rusbyi.

The species *N. Rusbyi,* collected in Bolivia by Dr. O. E. WHITE, was sent to the Bussey Institution of Harvard University rather recently; hence, its genetic history is short. It produces partially fertile hybrids reciprocally with *N. Tabacum,* hybrids which resemble the latter species rather closely (BRIEGER 1927, 1928). It failed to produce hybrids with *N. Langsdorffii* reciprocally, and as male on *N. rustica,* but it hybridizes freely in both directions with *N. glauca* and *N. tomentosa* (KOSTOFF). The hybrids with *N. glauca* are vigorous but sterile plants, intermediate in character; the hybrids with *N. tomentosa* are not yet old enough to describe.

N. sylvestris.

Attempts to cross *N. sylvestris* with the *alata* and *longiflora* groups, and with *N. acuminata, N. glutinosa, N. trigonophylla, N. suaveolens, N. nudicaulis* and *N. rustica* by EAST and by CHRISTOFF have failed.

The species is a *N. Tabacum* relative. In fact, it probably may be accepted as the most specialized form of the Tabacum section. It forms vigorous (120 per cent), partially fertile hybrids with *N. Tabacum,* which closely resemble the latter species (BELLAIR, EAST, GOODSPEED and CLAUSEN, MALINOWSKI, ALLARD, MALLOCH and MALLOCH). CLAUSEN and KOSTOFF have made reciprocal combinations with *N. tomentosa,* which are vigorous but sterile; and CHRISTOF has used it as male on *N. glutinosa,* obtaining two weak intermediate hybrid plants. One may predict that it will cross with *N. Rusbyi.*

N. tomentosa.

N. tomentosa, though excluded from the genus and recorded as *Lehmannia tomentosa* SPR. by COMES, is clearly a *Nicotiana* related to *N. Tabacum.* Unfortunately it has been used but little in hybridization, the only record with incompatible species being a failure on *N. Langsdorffii,* reported by KOSTOFF. The successful reciprocal crosses with *N. glauca, N. Rusbyi,* and *N. sylvestris* (KOSTOFF) have already been described. In addition, GOODSPEED and CLAUSEN have successfully used *N. tomentosa* as the male parent in crosses with *N. trigonophylla, N. nudicaulis,* and *N. Tabacum* (also KOSTOFF). One suspects that *N. tomentosa* will cross with *N. glutinosa.*

N. trigonophylla.

Many attempts to cross *N. trigonophylla* have been made. It has been tried (usually reciprocally) with all of the species on our tabular list, except *N. Rusbyi* and *N. attenuata,* with only two successes. CHRISTOFF (also GOODSPEED and CLAUSEN) produced the hybrid *N. nudicaulis* × *N. trigonophylla.* The seeds showed half-normal germination. The sterile, vigorous plants (110 per cent) were an intermixture of the characters of the parents, but were more nearly like *N. nudicaulis.* GOODSPEED and CLAUSEN have obtained *N. trigonophylla* × *N. tomentosa. N. trigonophylla* will probably give partially (or completely) fertile hybrids with *N. Palmeri;* but the cross has not been attempted.

N. suaveolens.

N. suaveolens, said to be the only old-world representative of the genus, exhibits a somewhat general compatibility with other species, — particularly when used as mother plant. The only case where failure is recorded when it was used as maternal parent and success when it was used as pollen parent is the combination *N. rustica* × *N. suaveloens*, recorded by GÄRTNER, which gave weak dwarfs whose characters are not described. GÄRTNER's reciprocal combinations with *N. glutinosa* and *N. paniculata* have already been described. Later workers have produced only the hybrid *N. suaveolens* × *N. glutinosa*, CHRISTOFF obtaining five vigorous intermediate plants. In addition, vigorous hybrids, resembling *N. Bigelovii*, of *N. suaveolens* × *N. Bigelovii* have been produced by GÄRTNER, GOODSPEED and CLAUSEN, EAST and CHRISTOFF; while practically identical hybrids of the reciprocal cross have been produced by GÄRTNER, GOODSPEED and CLAUSEN, EAST, and CHRISTOFF. GÄRTNER also obtained the hybrid *N. suaveolens* × *N. Tabacum*. Besides these combinations, *N. suaveolens* has given hybrids with *N. Langsdorffii*, *N. longiflora*, and *N. plumbaginifolia*, as already described. The closest relative to *N. suaveolens*, if the genetic evidence is sound, is *N. Bigelovii*.

N. attenuata.

N. attenuata has hardly been tested sufficiently to relate it to any of the other species. There are no recorded attempts to use it as female. As pollen parent it has failed with *N. Langsdorffii* and *N. Sanderae* (KOSTOFF). A single huge hybrid, *N. rustica* × *N. attenuata* (*N. californica* NAUD.) was obtained by NAUDIN. It was 170 cm. in height and intermediate. KOSTOFF has obtained seeds with non-viable embryos in this combination. KOSTOFF's attempts at crossing were made with the plants raised from the seeds of the GRAY Herbarium sheet (labeled *N. viscosa*,) on which the chromosome count was $N = 24$.

N. Bigelovii.

N. Bigelovii shows relationships with *N. glutinosa*, *N. suaveolens*, *N. Tabacum*, and with *N. nudicaulis*. It failed reciprocally with the *alata* group, with the *longiflora* group, and with *N. acuminata*, *N. sylvestris*, and *N. trigonophylla*, with one exception. EAST obtained the combination *N. Langsdorffii* × *N. Bigelovii*, the vigorous plants being very like the pollen parent.

The reciprocal hybrids with *N. glutinosa* and *N. suaveolens* have already been mentioned. In addition, GOODSPEED and CLAUSEN, and CHRISTOFF have produced the combination *N. Bigelovii* × *N. nudi-*

caulis. In CHRISTOFF's experience, the germination of the hybrid seeds was only a little over one-tenth normal; but the plants grew vigorously. The type was strictly intermediate, although perhaps the size of the flowers approached more closely to *N. nudicaulis*. GOODSPEED and CLAUSEN have produced the reciprocal. The hybrid with *N. Tabacum* is probably the easiest to make. Made with *N. Tabacum* as male, it has been produced by GÄRTNER (using var. *quadrivalvis*) GOODSPEED and CLAUSEN, EAST, and CHRISTOFF; made with *N. Tabacum* as female, it has been produced by EAST (CHRISTOFF obtained no viable seeds). The hybrids in the experience of EAST are vigorous (120 per cent), sterile, and strictly intermediate; though GÄRTNER thought they stood somewhat closer to *N. Tabacum.*

N. nudicaulis.

N. nudicaulis, in so far as it has been tested, shows rather distant relationship with *N. Bigelovii*, the most vigorous hybrids having been obtained with this species, as mentioned above. CHRISTOFF's hybrids of *N. nudicaulis* × *N. trigonophylla* were also rather vigorous, however. The only other hybrid that he obtained, *N. glutinosa* × *N. nudicaulis*, was weak. GOODSPEED and CLAUSEN have been successful with the combinations produced by CHRISTOFF, and in addition have obtained *N. nudicaulis* × *N. glauca*, *N. nudicaulis* × *N. tomentosa*, and *N. nudicaulis* × *N. Bigelovii*. Failures are recorded as female with *N. alata*, *N. Langsdorffii*, *N. paniculata*, *N. sylvestris*, *N. suaveolens*, *N. rustica*, and *N. Tabacum*; and as male with *N. alata*, *N. Langsdorffii*, *N. acuminata*, *N. glauca*, *N. paniculata*, *N. trigonophylla*, *N. suaveolens*, *N. rustica*, and *N. Tabacum.*

N. rustica.

N. rustica, is most closely related to *N. paniculata* with which, reciprocally, it forms partially fertile *rustica*-like hybrids. As pollen parent, it has been used on all of the other species on our list with the exception of *N. Rusbyi*, *N. tomentosa*, and *N. attenuata*, without result. As maternal parent it formed a hybrid with *N. attenuata*, if NAUDIN's *N. californica* is to be taken as this species; but this is a questionable determination. The hybrids made with pollen from *N. Langsdorffii*, *N. Sanderæ*, *N. paniculata*, *N. suaveolens*, and *N. Bigelovii*, have already been mentioned. In addition, GÄRTNER, EAST, and CHRISTOFF have obtained *Tabacum*-like hybrids with the pollen of *N. Tabacum. N. rustica* failed to produce hybrids with pollen of *N. alata, N. longiflora, N. plumbaginifolia, N. acuminata, N. glutinosa N. Rusbyi*, and *N. trigonophylla.*

N. Tabacum.

N. Tabacum hybridizes very easily, partly, no doubt, because so many attempts have been made to utilize it. It has been used in at least one combination with all of the eighteen species being considered, with complete failure only in connection with *N. plumbagini folia*, and *N. trigonophylla.* Judging by the partially fertile hybrids it forms with *N. sylvestris, N. glutinosa, N. Rusbyi*, and *N. tomentosa*, these are the species to which it is most closely related. (See table II, page 268).

Cytological behavior of F_1 inter-specific hybrids and certain back crosses

According to GOODSPEED (1923), SACHSOWA (C. R. Soc. Sc. Varsovie, 1917) and later WOYCICKI (Dis. Biol. Arch. Soc. Sc. Varsoviensis, 1921) published accounts of the cytological behavior of hybrids between *N. Tabacum* (24) and *N. Sylvestris* (12). I have not been able to obtain either of these papers, which appear to be the first discussions of the cytology of *Nicotiana* hybrids. GOODSPEED states that neither investigator appeared to realize that the parental species differed in chromosome number. WOYCICKI, however, spoke of twelve large and twelve small chromosomes as being paired. SACHSOWA believed that the so-called "Drosera scheme" did not apply, but figured bivalent and univalent chromosomes behaving in the manner later described by GOODSPEED.

GOODSPEED (1923) described and figured the cytological phenomena exhibited by F_1 hybrids of *N. sylvestris* × *N. Tabacum.* Twelve bivalent and twelve univalent chromosomes were present at the heterotypic metaphase of both pollen and embryosac mother cells

The first division of the pollen mother cells is said to consist of two successive stages, one involving the separation of the bivalents and the other the division of the univalents. Though the situation was not altogether clear, it was thought that both halves of univalent chromosomes when dividing near one pole would be included in one daughter nucleus, and therefore that daughter nuclei would be formed with variant numbers of chromosomes.

TABLE II. — COMPATIBI

		9 *N. alata*	9 *N. Langsdorffii*	9 *N. Sanderae*	10 *N. longiflora*	10 *N. l. plumb.*	12 *N. acuminata*
N. alata	9	—	H1345	H45	H34 x5	x5	x45
N. Langsdorffii	9	H1345	—	H45	x45	x5	x5
N. Sanderaae	9	H45	H4	—			x4
N. longiflora	10	H345	x5	H7 x45	—	H45	x5
N. l. plumb.	10	H25 w4	H5	x4	H245	—	x5
N. acuminata	12	w4p5	x15	x45	w4p5	w4p5	—
N. glauca	12	H6	H16	H6			
N. glutinosa	12	x45	x1w5	p45	p5	p5	p15x4
N. paniculata	12	H4 w5	H123 456	H5 w4	p4w5	w5	p4x5
N. Rusbyi	12		p6				
N. sylvestris	12	x4p5	x45	x4p5	p4x5	x5	x45
N. tomentosa	12						
N. trigonophylla	12	x4p5	x4w5	p5	x4p5	x4p5	x5
N. suaveolens	16	p4w5	H1 x45	w4x5	H45	H5 p1	w4
N. attenuata? possibly *N. viscosa*	24						
N. Bigelovii	24	p45	p14w5	x4p5	p4w5	w5	p5
N. nudicaulis	24	p5	p5				
N. rustica	24	w4	H456 w1	H56	p5x6	p5	x4p5
N. Tabacum	24	H1345	H4 p1	H56	H4 w5	w45	H3 p1x5

Legend: x, no stimulation; p, parthenocarpy; w, weak embryos; H, hybrids. Cr
(EAST & HAYES 1912 and unpublished), (5) CHRISTOFF (1928), (6) KOSTOFF

Later GOODSPEED and CLAUSEN (1927*a*) published a more extended account the of cytological features exhibited by these hybrids. The new data established several important facts. A well organized equatorial plate always appears. From other experiences with hybrids that exhibit pairing and with hybrids that do not exhibit pairing, the authors conclude that pairing is essential for the formation of

S NICOTIANA SPECIES

12 *N. paniculata*	12 *N. Rusbyi*	12 *N. sylvestris*	12 *N. tomentosa*	12 *N. trigonophylla*	16 *N. suaveolens*	24 *N. attenuata* ? possibly *N. viscosa*	24 *N. Bigelovii*	24 *N. nudicaulis*	24 *N. rustica*	24 *N. Tabacum*
H4 x5		x45		x5	x45		x45	x5	x45	H4 x5
x145	x6	x5	x6	x45	x145	x6	H4 x15	x5	x1456	x156
		x4			x4	x6	x4		x6	x4
x5		x45		x45	x45		x45		x56	x5
p5		x5		x5	x45		x5		x5	w4x5
x145		x45		x45	p1x45		x45	p1x5	x15	p145
x16	H6		H6	x56		x6	x5	x5	x6	H136
w14p5		H35		x45	H1 p5		H15 w4	H35	x145	H134 p5
—		x4p5		p45	H1 w45		H14 p5	p5	H134 p5	H14 p5
	—		H6							H8
x45		—	H36	x45	x45		x45		x45	H345
	H6	H6	—							
p45		x45	H3	—	x4p5		x4p5	p5	x4p5	p45
H1 p4x5		x45		x45	—		H1345	x5	x145	H1 w45
						—				
w15p4		x4w5		x45	H135		—	H35	w1x45	H1345
x5		x5	H3	H35	p5		H3	—	p5	w5
H1345	x6			p5x6	H1 w5	H1 w6	H14 p5	p5	—	H145
w4x5	H8	H345	H36	x45	x14p5		H4 x1p5	p5	p1x5	—

Pre-Mendelian hybridizers, (2) Lock (1909), (3) Goodspeed & Clausen, (4) East
) Malloch & Malloch (1924), (8) Brieger (1927, 1928), (9) Clausen (1928).

a characteristic equatorial-plate stage. Twenty-four chromosomes are always present at diakinesis and first metaphase, twelve large bivalents and twelve small univalents. At first metaphase, varying numbers of the univalents are to be found at the equatorial plate, others being seen lying near one or the other of the poles. The fate of the univalent chromosomes was determined by counting the number observed outside of the daughter nuclei at interkinesis, it being assumed that these chromosomes make up the total number not included. A count of 140 pollen mother cells showed 0, 1, 2 and 3 chromosomes in the plasma with frequencies 94, 39, 6, and 1 respectively. A count of the chromosomes at second metaphase led to the conclusion that the univalents are distributed at random.

The F_1 plants of this hybrid are sterile *inter se*, but exhibit some fertility with the pollen of both parental species. Several of these back-crossed plants have been investigated cytologically. Two plants strikingly like the plants of the F_1 generation exhibited 12 bivalents and 12 univalents at first metaphase. Their characters, however, indicate a replacement of some of the "sT" pairs of chromosomes by "ss" pairs. An extreme semi-dwarf plant abnormal in appearance had 12 bivalents and 7 univalents at first metaphase. In contrast, a *sylvestris*-like plant had only 12 bivalents. Another *sylvestris*-like plant, which nevertheless differed from that species in having shorter flowers and stamens and somewhat different leaves, had 12 bivalent and 1 univalent chromosomes. There were two other plants exhibiting this chromosome number, which had different characteristics. One plant showed 12 bivalents and 2 univalents.

The next *Nicotiana* hybrid to be studied cytologically (GOODSPEED CLAUSEN and CHIPMAN 1926) in detail is that between *N. rustica* (24) and *N. paniculata* (12). EAST (1921) tentatively reported 24 haploid chromosomes for *N. paniculata* from only two slides in which the exact phases appearing could not be determined. This determination was wrong. The species has but 12 chromosomes haploid. The situation, therefore, is the same as in the previous cross; it is a union between a 24-chromosome species and a 12-chromosome species.

The meiotic divisions in the F_1 generation are practically the same as in the *sylvestris-Tabacum* hybrid. The "Drosera scheme" is followed. There are 12 bivalents and 12 univalents. The bivalents are regularly distributed to the daughter nuclei. The univalents exhibit a random distribution, though a simulated division is sometimes seen. The homeotypic division is rather regular, though a few chromosomes often lag. Normal tetrads are formed, and the pollen grains sometimes function.

A back cross with *N. paniculata* pollen yielded an extremely variable population of 44 plants. Ten of the plants which appeared to be the most fertile of this back cross, were again crossed with *N. paniculata*. Only three yielded seed, and only one yielded seed in abundance, indicating that this type of population is less fertile than the F_1 generation. Of the original back cross, the chromosome numbers were determined for 34 plants. All had 12 bivalents. In addition there were 2, 3, 4, 6, 8, 9 and 12 univalents with the following frequencies; namely 1, 1, 4, 8, 4, 2 and 14. The prevalence of plants with 12 bivalents plus 12 univalents led to a suspicion that early division of the univalents was occurring, but the evidence did not support this idea.

The above data are believed by the authors to indicate that the univalents are distributed at random and appear in the gametes in frequencies from 0 to 12, corresponding to the frequencies of the coefficients of the terms of $(a + b)^{12}$. It is also assumed that there is random survival of the gametes produced, though it is agreed that such an assumption is difficult to reconcile with the high degree of abortion exhibited in the pollen of the F_1 generation. The assumption is indeed impossible to reconcile with the pedigree culture data; and since the cytological data reported do not warrant such a statistical conclusion, it would seem to be an unwise postulate.

The same F_1 *rustica* (*brasilia*)-*paniculata* plant used in the above experiments was backcrossed to *N. rustica brasilia*. Among the 15 plants raised to maturity, there was great variability. The prevailing type was *rustica*, though the plants did not resemble the *brasilia* variety. The chromosome situation was determined in the case of 11 plants; 7 exhibited 18 bivalents and 6 univalents, while 4 exhibited 20 bivalents and 4 univalents.

The cytological behavior of the F_1 of this cross and its reciprocal has also been studied by CHRISTOFF (1928), and by another worker in my laboratory whose work is not yet published (W. R. SINGLETON). They substantiate the general description of GOODSPEED and CLAUSEN. SINGLETON is making a cytological study of a straight F_2 population obtained by selfing F_1 plants. The *rustica* parent used belonged to variety *humilis*; yet 4 completely fertile F_2 plants showing 24 bivalents, resemble variety *brasilia*. Among the other plants, it may be noted that at least 4 have 36 bivalent chromosomes, and are almost completely fertile when selfed. At least 2 of these plants set seed when pollinated by *N. rustica, but are completely sterile to the pollen of N. paniculata.*

A third hybrid of this type has been studied in some detail by Brieger (1927, 1928). In the first paper, it is merely stated that F_1 hybrids between *N. Tabacum* (24) and *N. Rusbyi* (12) show 12 bivalents and 12 univalents. The possibility of *N. Tabacum* having arisen as a cross between *N. sylvestris* and *N. Rusbyi* is also discussed, but is dismissed because the existing varieties of *N. Tabacum* show no such combination of genes.

Brieger's second paper (1928) gives an extended account of the genetics and cytology both of the F_1 generation and of back crosses to *N. Tabacum.* As to the genetics of the cross, it will be sufficient to say that the dominant character of the *N. Tabacum* contribution is shown. In the cytological observations of the F_1 generation, it is shown that the "Drosera scheme" is followed in the development of the pollen mother cells. The development of the embryo sac mothers cells is also figured; and while it is probable that the same scheme obtains, the matter is not so clear. Nevertheless it is obvious that the bivalents (presumably 12) are arranged in an orderly manner at the equatorial plate, while the univalents are distributed irregularly over the spindle.

There was complete pollen sterility. Yet in back crosses a slight degree of fertility was exhibited with *N. Rusbyi* pollen and perhaps 4 per cent of the normally expected fertility was exhibited with *N. Tabacum* pollen.

The number of chromosomes of the F_1 gametes presumably varied between 12 and 24, since these numbers of pairs were observed in back crosses with *N. Tabacum* pollen. The rest of the 24 *Tabacum* chromosomes of course remained unpaired. Such was the case in 21 plants investigated, 24 chromosome elements always being present at heterotypic methaphase.

Two exceptional back cross plants were also investigated. One possessed 60 chromosomes, evidently formed by the union of an F_1 gamete containing 36 chromosomes with a *Tabacum* gamete containing 24 chromosomes. The other plant possessed 55 chromosomes. The lack of certain F_1 chromosomes in this plant was correlated with the absence of certain *Rusbyi* characters. Both of these plants showed trivalents, bivalents, and univalents at the heterotypic metaphase. But the trivalent combination was not always sufficiently strong to prevent a breaking apart into a bivalent and a univalent.

Other "Drosera scheme" hybrids are *glutinosa* (12) — *Tabacum* (24) (CLAUSEN and GOODSPEED, 1925) based on circumstantial evidence; *longiflora* (10) — *alata* (9), and *tomentosa* (12) — *Tabacum* (24) GOODSPEED and CLAUSEN 1927*a*); and *longiflora* (10)—*Sanderae* (9, or 8 according to CHRISTOFF) (CHRISTOFF 1928).

Dr. D. KOSTOFF of my laboratory has unpublished observations which show that *Tabacum* (24)—*glauca* (12), *glauca* (12)—*Langsdorffii* (9), and *Tabacum* (24)—*Sanderae* (9) show the *Drosera* type of behavior. In the first of these crosses, the meiotic divisions are very irregular, non-disjunction being observed frequently. The plants are sterile *inter se* and in back crosses. In the second cross, the meiotic divisions are often retarded, and diads and monads are formed by the suppression of division phases. The hybrids are fertile to the pollen of *N. Langsdorffii*. They are peculiarly characterized by the formation of tumors. The third cross is also sterile *inter se*, in back crosses, and to the pollen of *N. Langsdorffii*.

Dr. GOODSPEED permits me to report that he has unpublished data showing *Drosera* type behavior on the following crosses, in addition to those reported in previous papers:

N. alata × *N. longiflora*, *N. longiflora* × *N. alata*, *N. glauca* × *N. Tabacum*, *N. Tabacum* × *N. glauca*, *N. glutinosa* × *N. nudicaulis*, *N. rustica* × *N. solanifolia*, *N. nudicaulis* × *N. trigonophylla*, and *N. nudicaulis* × *N. tomentosa*. In addition, the following hybrids exhibit *Drosera* type behavior with some variation: *N. paniculata* × *N. rustica*, *N. glutinosa* × *N. Tabacum*, *N. Tabacum* × *N. glutinosa*, and *N. Tabacum* × *N. glauca*.

A second type of cytological behavior in *Nicotiana* hybrids was first described by CHRISTOFF (1928). It is a variable amount of pairing in which the number of gemini seldom reach the full haploid number of the parental species having the smaller number of chromosomes. Frequently in these cases some of the univalents pass to the poles before the distribution of the bivalents begins. Sometimes there is precocious division giving plates having an increased number of chromosomes at late stages of the first meiotic division. This divisional behavior CHRISTOFF found in *N. Tabacum* × *N. alata* and *N. Tabacum* × *N. Sanderae*. GOODSPEED (unpublished data) has found similar cases in the following crosses: *N. trigonophylla* × *N. tomentosa*, *N. nudicaulis* × *N. Bigelovii*, and *N. Bigelovvii* × *N. nudicaulis*.

In other hybrids there is no conjugation at meiosis. GOODSPEED and CLAUSEN (1927) first described such cases in this genus for the hybrids *N. Bigelovii* × *N. suaveolens* and *N. Bigelovii* × *N. glutinosa.* The peculiarities of these cases appear to be (*a*) a rapid succession of the first division phases, (*b*) great irregularity of chromosome distribution, (*c*) multipolar spindles becoming bipolar at late stages, and (*d*) distorted chromosomes and other nuclear abnormalities. CHRISTOFF (1928) found a similar state of affairs in the crosses: *N. paniculata* × *N. Langsdorffii, N. suaveolens* × *N. glutinosa,* and *N. suaveolens* × *N. Bigelovii.* Dr. GOODSPEED (unpublished) finds that the same situation obtains in the following list of hybrids: *N. paniculata* × *N. Langsdorffii, N. paniculata* × *N. glauca, N. glutinosa* × *N. glauca, N. sylvestris* × *N. tomentosa, N. suaveolens* × *N. solanifolia, N. nudicaulis* × *N. glauca, N. Bigelovii* × *N. solanifolia* and *N. Bigelovii* × *N. Tabacum.*

It is rather notable that in so far as these investigations overlap, there is little difference of opinion on the behavior of the hybrids concerned. The only outstanding case is that of *N. Tabacum* × *N. alata* in which CHRISTOFF finds a variable amount of pairing up to eight pairs, while GOODSPEED finds no conjugation whatever.

The behavior of hybrids between species having the same chromosome number is yet to be considered, although the crosses in which no conjugation occurs have already been listed. Normal pairing takes place in all combinations of the *alata* group, in reciprocals between the supposed species *N. longiflora* and *N. plumbaginifolia,* in *N. paniculata* × *N. solanifolia* (GOODSPEED unpublished), and in *N. glauca* × *N. Rusbyi* (KOSTOFF unpublished). There is also the cross between *N. Tabacum* and *N. rustica,* about which there is some difference of opinion. CHRISTOFF (1928) was uncertain whether more than a few loose gemini were ever found; KOSTOFF (unpublished), on the other hand, has observed normal pairing. I have examined the slides of both of these workers and can vouch for the correctness of each. Both species are variable; and it is probable that complete conjugation occurs in some cases and not in others. KOSTOFF found that the actual chromosome distribution was very irregular. Non-disjunction was often observed. Moreover the second meiotic division was omitted in from 3—5 per cent of the cases studied, giving diads with the somatic number of chromosomes. The hybrids were fertile to the pollen of *N.*

Tabacum. N. rustica also gave plants when pollinated with pollen from the F_1 plants.

Besides the back crosses of *Tabacum-sylvestris, paniculata-rustica,* and *Tabacum-Rusbyi,* with one or both parents, the significant features of which have already been described, Dr. KOSTOFF (unpublished) has made some interesting studies of a similar type.

The hybrid *N. rustica* × *N. Tabacum sanguinea* was crossed back with *N. Tabacum sanguinea.* In 15 plants studied, the first metaphase of the pollen mother cells showed 36—38 chromosomes. In 4 of these plants the somatic number of chromosomes was 70—72. One plant with 32 chromosomes at first metaphase, had only 53 somatic chromosomes. In the pollen mother cells, univalents, bivalents, and trivalents were observed. These cells form tetrads, triads, and diads most frequently; yet monads and even octads occur. The plants usually show some fertility when selfed, when used as females with *N. Tabacum,* and when used as males with *N. rustica.* One plant of a second back cross to *N. Tabacum* was found to have 30 chromosomes in the first metaphase of the pollen mother cells. The cytology of the cross *N. rustica* × (*N. rustica* × *Tabacum*) is similar to the above. The plants appear to be sterile to their own pollen but are fertile as males on *N. rustica.*

The hybrid *N. glauca* × *N. Langsdorffii* has been found to be fertile with the pollen of *N. Langsdorffii, N. Sanderae* and *N. alata,* but sterile with *N. glauca.* Of the plants of the back cross with *N. Langsdorffii,* 3 have 30 chromosomes, 1 has 32 chromosomes, and 1 has 21 chromosomes in root-tip slides. In the last plant, 10 chromosomes appear at first metaphase of the pollen mother cells. Of these, 2 are apparently trivalents, 7 are bivalents, and 1 a univalent, making 21 in all.

A single plant has been studied of another rather interesting combination, *N. Tabacum* × (*N. Langsdorffii* × *N. Sanderæ*). It has 33 chromosomes in the somatic tissues. In the first metaphase of the pollen mother cells, 26 chromosomes appear, 7 being larger than the other 19. If one assumes the *N. Tabacum* contribution to be 24 chromosomes, then since 9 bivalents are found in *N. Tabacum* × *N. Sanderæ* (KOSTOFF) and no conjugation is observable in *N. Tabacum* × *N. alata* (GOODSPEED), it may be that here *N. Sanderae* has contributed 7 chromosomes and *N. alata* has contributed 2 chromosomes.

Perhaps the most unusual cross is one involving three chromosome numbers, 24, 12, and 9. This is a cross between an F_1 plant of *N. rustica* × *N. paniculata* with *N. Langsdorffii*. It has been possible thus far to study only two plants. One has 24 chromosomes in the somatic tissues with 1 trivalent group appearing in the first metaphase of the pollen mother cells. The other has 32 somatic chromosomes, with 5 trivalent groups appearing in the same phase.

Origin of new polyploid forms through hybridization

Several investigations having shown that the chromosome numbers in a given genus are examples of arithmetical progression (*Triticum, Rosa, Chrysanthemum*), it has been pointed out that successive chromosome doubling may be a factor in the origin of species. WINGE (1917), however, has called attention to the fact that successive chromosome doublings would give rise to geometrical series; and has suggested as an alternative hypothesis a chromosome doubling following hybridization.

COLLINS and MANN (1923) suggested that irregular chromosome distribution in interspecific hybrids in which the chromosomes do not pair at meiosis is a method by which this might occur. CLAUSEN and GOODSPEED (1925) have added three other methods: (*a*) chromosome doubling immediately after fertilization, (*b*) bud variation in the F_1 generation and (*c*) hybrids of tetraploid representatives of two species. Naturally these four schemes concern only the formation of tetraploids.

The present state of knowledge hardly warrants our being influenced very strongly by the idea of arithmetical series among the species of a particular genus. All sorts of other ways of obtaining new forms in which the chromosome numbers are not in such series are imaginable. But it is true that a tetraploid formed in any one of these four ways should be fertile because it would contain two sets of the chromosomes of each species. Moreover, such types have actually been discovered.

The first case is a tetraploid *glutinosa* (12)—*Tabacum* (24) hybrid ($n = 36$) recorded by CLAUSEN and GOODSPEED (1925). Reciprocal hybrids of these species were easily obtained; but seed production was limited and the hybrids weak, except when the Cuban variety of

N. Tabacum was used. Usually all F_1 plants were found to be completely sterile; but in 1922 CLAUSEN and GOODSPEED obtained three plants from the cross *N. glutinosa* × *N. Tabacum purpurea*. One was probably haploid and one was completely sterile. The third produced quantities of seed when selfed. This plant was a rather weak specimen about 60 cm. high, with small leaves. The bilabiate flowers resembled *N. glutinosa* but had the red color of the other parent. The selfed seed of this plant yielded a strictly uniform population of 65 plants which were very much like those of an F_1 population of *N. Tabacum purpurea* × *N. glutinosa* which was growing in the garden at the time. Cytological examination of several of these plants showed that each exhibited 36 bivalents at the first metaphase of the pollen mother cells, in the loose type of conjugation which CLAUSEN terms the Boreale type. The divisions were fairly regular; though minor irregularities, such as precocious splitting, lagging, and microcyte formation, were observed. The evidence, therefore, was conclusive that here was a new tetraploid species. It has been called *N. digluta*. CLAUSEN (1928) has recently reported that when *N. digluta* is crossed reciprocally with either of the species that entered into its makeup. the "Drosera scheme" of conjugation is exhibited by the F_1 hybrids at meiosis. *N. digluta* × *N. glutinosa* forms 12 bivalents and 24 univalents; while *N. digluta* × *N. Tabacum* forms 24 bivalents and 12 univalents, — as is to be expected from the chromosome contributions made to *N. digluta* from *N. glutinosa* and *N. Tabacum*. Dr. GOODSPEED informs me that *N. digluta* produces hybrids with the pollen of *N. solanifolia*; but there is no chromosome conjugation in the first metaphase of their pollen mother cells.

EGHIS (1927) has obtained a tetraploid originating in a manner different from the so-called *N. digluta*. The cytological work was done by RYBIN (1927).

Two plants were raised from two different crosses of *N. rustica aurea* × *N. Tabacum*. Each plant was slightly fertile, producing a few viable seeds when the flowers were unguarded. Each exhibited 48 chromosomes in the somatic cells. Plants with 48 chromosomes, identical with the maternal species, were also obtained both in this combination and the reciprocal. Whether this was the result of induced apogamy or polyembrony, or parthenogenesis with subsequent doubing of the ch romosomes, is unknown.

From the reciprocal cross, *N. Tabacum* × *N. rustica,* a single hybrid plant was obtained which proved to be a triploid. Presumably 48 chromosomes were contributed by *N. Tabacum* and 24 chromosomes by *N. rustica.* Although this plant was sterile to its own pollen, it produced a series of hybrid forms when pollinated with pollen from *N. Tabacum, N. rustica, N. sylvestris, N. alata grandiflora, N. Sanderae, N. Langsdorffii, N. glauca,* and *N. paniculata.* When crossed with pollen from *N. rustica,* a fertile plant was obtained which had 96 chromosomes in the somatic tissues. At heterotypic metaphase, 48 bivalents were found. One of the most interesting features of this plant is that although the pollen was apparently normal and could be caused to germinate almost perfectly on artificial media, it was more efficacious when used on *N. rustica* than when used on *N. Tabacum.*

Certain phases of BRIEGER's work (1928) with *Tabacum—Rusbyi* hybrids may perhaps be mentioned in this connection, although he obtained no tetraploids. The cross *N. Rusbyi* × *N. Tabacum* yields but few seeds which germinate poorly; the reciprocal, on the other hand, yields many seeds which germinate readily and produce vigorous plants. The F_1 plants, which follow the "Drosera scheme" in gamete formation, are sterile to their own pollen, but cross readily with both parents. The back cross with the *N. Rusbyi* pollen, yields weak plants which are with difficulty brought to flower. Their pollen mother cells degenerate early. The back cross (*N. Tabacum* × *N. Rusbyi*) × *N. Tabacum,* on the contrary, is quite vigorous. A cytological examination was made of 21 plants of such a population. Each showed 24 chromosome elements at first metaphase, — some being bivalents and some univalents. Since the gametes of the F_1 plants possess from 12 to 24 chromosomes, it is assumed that all chromosomes contributed by the F_1 mother plant pair with chromosomes from *N. Tabacum,* leaving any extra chromosomes of *N. Tabacum* unpaired. The evidence tended to show that no plant had more than 48 chromosomes as the diploid number.

Two other plants were exceptional in having more than the expected number of chromosomes. One plant possessed 60 chromosomes. It evidently originated through the union of an F_1 gamete having the full 36 chromosomes with a gamete from *N. Tabacum* having 24 chromosomes, such F_1 gametes having been observed to originate through a spindle-fusion at the second maturation division which resulted in diads. Moreover, the back-crossed plant itself behaved similarly at the second maturation division.

The second plant had about 55 chromosomes. It is assumed to have had an origin similar to that of the other, but with several *Rusbyi*-chromosomes omitted. Absence of certain *Rusbyi* characters encouraged such a view.

Both plants exhibited trivalents, bivalents, and univalents at the first meiotic division. The union of the trivalents was not strong enough to prevent their separation into a bivalent and a univalent element.

While these plants were only slightly more fertile than those of the F_1 generation, there seems to be no reason, as BRIEGER observes, why one should not accept this type of cytological behavior as a possible means of obtaining new races. In BRIEGER's opinion, the five principal modes of chromosomal increase through irregular meiosis are: (*a*) unsymmetrical chromosome distribution, (*b*) fusion of spindles at the second maturation division, (*c*) premature onset of the second maturation division whereby the reduction division is suppressed, (*d*) failure of reduction, and (*e*) fusion of two metaphases in the first meiotic division (cf. KARPETSCHENKO's experience with the hybrid *Raphanus sativus* × *Brassica oleracea*).

The behavior of partially fertile hybrids

Of the considerable series of *Nicotiana* hybrids which show some slight fertility, only three have been studied intensively. These three hybrids are *rustica-paniculata*, which has the honor of being the first plant hybrid to be thoroughly investigated, *sylvestris-Tabacum* and *Tabacum-Rusbyi*. The principal post-Mendelian researches on these hybrids have been made by EAST, GOODSPEED and CLAUSEN, and BRIEGER, respectively; though the work of BRIEGER published thus far has dealt primarily with the cytology of the material. All three of these hybrids, it is interesting to note, are unions of 12-chromosome species and 24-chromosome species.

The cross between *N. paniculata* and *N. rustica* is perhaps the most interesting of the species hybrids, not only because of its historical significance, but also because the F_1 plants are somewhat fertile *inter* *se*, which is not the case with *sylvestris*—*Tabacum* or *Tabacum*—*Rusbyi*. An analysis of six years' work in connection with these hybrids was made in 1915 (East 1915), but the complete reports were not published until 1921, (East 1921). The significant feratures may be outlined as follows:

Reciprocal crosses were made between *N. rustica humilis* and *N. paniculata*. Comparative studies were also made with crosses between *N. rustica scabra* and *N. paniculata*, and between *N. rustica brasilia* and *N. paniculata*. The results were so similar that only the first study need be considered. The two parental species, which had given extremely uniform populations of progeny when selfed, produced F_1 populations as uniform as the parental populations. The F_1 plants, though they exhibited the influence of *N. paniculata*, resembled *N. rustica* much more closely. From one to six per cent of the female gametes of the hybrids were functional. It was impossible to determine accurately the percentage of viable male gametes formed from the pollen mother cells, but it was thought to be less than one-tenth of one per cent. The F_1 plants were as fertile *inter se* as they were in back crosses with either parent. As high as 4 per cent of the normal complement of seeds was obtained after self pollination, and as high as 60 per cent of these seeds germinated.

Extreme variation was exhibited by the F_2 plants. Practically no two plants were alike. A taxonomist engaged in classifying them would have been compelled to describe several different species. Most of the plants were *N. rustica* forms. The parental types were recovered once in every 100 or 200 plants; but the *paniculata* types recovered were *paniculata*-like in every particular, while the *rustica*-like plants were of every description. That is to say, there was a great gap between the few *paniculata*-like plants and those plants which resembled them most closely. If one set up an imaginary frequency distribution of possible recombinations of the characters in *N. rustica* and *N. paniculata*, on comparison the actual F_2 population would show a whole series of plants on the *rustica* side of the mean, and then complete absence of recombination types until extremes identical with *paniculata* were reached.

In the first experiment, in which 246 F_2 plants were raised, three plants appeared which were indistinguishable from pure *N. paniculata*. Four others stood closer to *paniculata* than to *rustica*. Two of the three *paniculata* segregates were as fertile as the true species, but one was completely sterile. Naturally, some suspicion arose as to whether these individuals had not arisen as volunteers, since traceable errors of this kind were found to occur about once in 2000 plants. The entire epxeriment was repeated, therefore, with similar results. In the second experiment in which 300 F_2 plants were raised, there was a single *paniculata* segregate which, curiously enough, was only about $^2/_3$ the size of a normal *paniculata*, though the size relations of all the organs were retained in their proper ratio. There seems to be no doubt, therefore, that the *paniculata*-like plants are true segregates.

The *rustica*-like segregates included all of the six forms described by COMES, although the small variety *humilis* had been used in the cross. Moreover, there were giant forms and dwarf forms, with all sorts of combinations of flower types and leaf types. The size of the flowers did not pass beyond the extremes of the species entering into the cross; but dwarf individuals were found with leaves one-fourth the size of the *rustica* parent, and giant individuals appeared with leaves 10 times the size of the *rustica* parent.

The fertility of the F_2 plants varied from a condition similar to that of the F_1 population, or perhaps somewhat more sterile than it, to practically complete fertility. In fact, 90 per cent of the plants produced greater complements of good pollen and viable seed than did the F_1 generation. None produced a proportion of good pollen equal to the pure species (about 98 per cent), but three plants of the F_2 generation had about 96 per cent morphologically perfect pollen. Naturally, it cannot be maintained from mere microscopical examination that all pollen grains which appeared to be good were functional. Nevertheless, there is no question but that a fertility approaching that of the pure species was attained by certain plants of the F_2 generation.

A considerable number of these F_2 plants were selfed and the descendants kept under observation for several generations. Fifty-one such families from among the *rustica*-type segregates were studied carefully; and two facts stood out impressively. First, the families were all rather high in fertility, and showed a low variation in fertility within each group. Several families, in fact, showed scarcely any discernible variation in fertility, producing seeds with almost the profusion of the pure *N. rustica*. Every ovule seemed capable of being fertilized, and the amount of abortive pollen was so small (from 3 to 5 per cent) one would hardly suspect the hybrid origin of the strain. Second, the uniformity of each population visualized as the relative homozygosity of each parental F_2 individual was much greater than was to have been expected in a uniformly viable population produced by orthodox Mendelian recombination. About 50 per cent of the F_2 plants produced populations in which the homozygosity was similar to that exhibited by supposedly pure varieties of *N. rustica* which had been collected from various sources. The fertile *paniculata*-like individuals produced populations as uniform as those produced by pure *N. paniculata*.

It was realized (EAST 1915) that the selective elimination of F_2 zygotes and the recombination of functional gametes that give different grades of fertility in F_2 could hardly be given an ordinary Mendelian interpretation, but since there was no evidence of selective fertilization, because the F_1 plants were as fertile *inter se* as in back crosses, it was suggested that the following hypotheses might be applied without assuming irregularities of chromosome distribution:

> "If (1) there is a group of chromosomes in each parent that cannot be replaced by chromosomes from the other parent; if (2) there is a group of chromosomes from each parent, a percentage of which may be replaced by chromosomes from the other parent but where functional perfection of the gametes varies as their constitution approaches that of the parental forms; if (3) there are other chromosomes that have no effect on fertility and therefore can promote recombinations of characters in the progeny of fertile F_2 plants; if (4) a naked male nucleus entering the normal cytoplasm of the egg in the immediate cross can cause changes in the cytoplasm that will affect future reduction divisions; if (5) this abnormally formed cytoplasm is not equitably distributed in the dichotomies of gameto-genesis in the F_1 generation; if (6) it follows from (4) and (5) that F_2 zygotes may be formed which are less perfect in their gamete-forming mechanism than those of the F_1 generation; and if (7) the heterotypic division of gametogenesis does not necessarily form two cells alike in their viability."

GOODSPEED and CLAUSEN (1916) published an interpretation of the results obtained in their work with *N. sylvestris-N. Tabacum* in which they used a conception practically the same as that given above in (1) and (2). This conception they called the Reaction System. The California botanists have made a very extended study of this cross. The first three papers (GOODSPEED 1913, GOODSPEED and AYRES 1916, and GOODSPEED and KENDALL 1916) deal with the problems of sterility involved. The Reaction System hypothesis was suggested in 1916 (GOODSPEED and CLAUSEN 1916) and was supported by extensive data in later papers (GOODSPEED and CLAUSEN 1917, 1922). This cross had previously been studied by BELLAIR (1913), MALINOWSKI (1916), and ALLARD (1919); but none of these writers had made any significant contribution to theory.

The F_1 plants of the cross *N. sylvestris* × *N. Tabacum* were uniform, vigorous plants in which the influence of *N. sylvestris* could be observed, but this influence was dominated by the contribution of *N. Tabacum*. The hybrids were sterile to their own pollen, but were fertile in back crosses, both to pollen of *N. sylvestris* and to that of *N. Tabacum*. When *N. sylvestris* pollen was used, the hybrids set about 1 per cent of the normal complement of seeds. The sesqui-hybrids thus obtained showed great diversity of type, although about 10 per cent of them resembled *N. sylvestris* very closely, and were almost completely fertile. A variety of evidence indicated that these plants were what they seemed to be, that is to say, pure *N. sylvestris* segregates. Similarly, the back crosses with *N. Tabacum* pollen gave populations of extremely variable sesqui-hybrids, approaching *N. Tabacum* in character, those approaching *N. Tabacum* most closely being the most fertile. The so-called Reaction System conception from which GOODSPEED and CLAUSEN interpreted these results is described by them as follows:

> "Developing the reaction system hypothesis, it would appear that, if the *N. Tabacum* and *N. sylvestris* systems display a high degree of mutual incompatibility, any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up between the *N. Tabacum* and *N. sylvestris* elements. If the admixture be relatively slight, the inharmonious elements may not greatly affect the workings of the reaction system and there would result individuals showing practically the entire set of characters of one or the other parent, and such individuals

> would be fully fertile. A slightly greater proportion of inharmonious elements in the reaction system would result in such profound disturbances in its functioning as to produce the abnormal individuals of various kinds which make up so large a proportion of the progeny from such parentage. When the proportions of inharmonious elements in the gametes become still greater, they fail to function at all. It is upon the formation of such nonfunctional gametes or the attempt to produce them, that the partial sterility of the hybrid depends, and since in this particular case these form by far the greater proportion of gametes, the hybrid is very nearly completely sterile."

Unfortunately, neither EAST nor GOODSPEED and CLAUSEN realized that there was a difference in chromosome number in the species used in the two crosses under consideration. It was assumed that both *N. rustica—N. paniculata* and *N. sylvestris—N. Tabacum* had 24 chromosomes as haploid numbers. It has since been determined that *N. paniculata* and *N. sylvestris* have 12 chromosomes as the haploid number, while *N. rustica* and *N. Tabacum* have 24 chromosomes as the haploid number, both *rustica-paniculata* hybrids and *sylvestris—Tabacum* hybrids exhibiting the "Drosera scheme" of conjugation at meiosis. As a matter of fact, however, the suggestions made regarding a hypothetical interpretation of the results need not be revised in any essential way to meet the new facts.

GOODSPEED and CLAUSEN (1922), studying three *sylvestris* derivative lines originating from back crosses of *sylvestris—Tabacum* to *sylvestris* have shown that one line is identical with normal *sylvestris*, and that another line contains *Tabacum* elements for which the plant was heterozygous. This demonstrates, of course, that certain *Tabacum* chromosomes are sufficiently homologous with certain *sylvestris* chromosomes that they may be exchanged without disturbing the *sylvestris*-like nature of the resulting product. The recombination in *paniculata—rustica*, as well as in *sylvestris—Tabacum*, therefore, may be pictured somewhat as follows.

One may assume that in the production of gametes in the *paniculata—rustica* cross there are 12 *Tabacum* chromosomes which conjugate with 12 *rustica* chromosomes, and that the elements of these pairs may pass at random to the daughter chromosomes, as at normal segregation. In addition, the extra *N. rustica* chromosomes are distributed at random. Nearly all of the resulting gametes are non-functional unless they approach either 12 or 24 in chromosome number. The greater likelihood of contributions from *N. rustica* accounts for the greater number of *rustica*-like progeny obtained. The elimination of so many intermediate type gametes explains the absence of F_2 plants which are similar to those of the F_1 generation or which approach *N. paniculata*. Presumably *paniculata*-like plants are formed only when two haploid gametes having 12 chromosomes unite, and presumably even this is impossible except when a considerable number of *paniculata* chromosomes are duplex. Whether or not the assumptions regarding cytoplasm (EAST's hypothesis) are needed, is questionable and must await further investigations, but they are not wholly improbable.

The Behavior of Fertile Hybrids

Many of the species of *Nicotiana* are polymorphic. *N. Tabacum* and *N. rustica*, the two species cultivated most widely, have probably given rise to the greatest number of types and to the most diverse types. But the variability of *N. longiflora*, of *N. paniculata*, of *N. trigonophylla*, and of *N. suaveolens* is quite high. The variant forms of the first five species are apparently all characterized by identical chromosome numbers and by quantitative variations in characters of the same general nature. In other words, there is no good reason for giving specific rank to their varieties. As to conditions in the Australian group, passing under the name *N. suaveolens*, we are ignorant. It is quite possible that several species exist having the same general traits, but which will be shown to be distinct from each other by genetic research. Dr. GOODSPEED informs me that he has recently had under observation a number of types from different regions of Australia. One proved to be distinct from "typical" *N. suaveolens*, and had about 20 chromosomes as the haploid number. The other was a form standing close to the ordinary type, but with increased vigor and cell size and a haploid chromosome number of 32.

There are, however, two groups of species, the components of which have the same chromosome numbers and which yield fertile hybrids in all combinations, that any taxonomist would be compelled to describe as distinct entities. The first group is the *Bigelovii* group. It consists of *N. Bigelovii, N. quadrivalvis* and its variety *multivalvis,* and *N. Clevelandii.* About all that can be said of these species is that they form a single genetic group, having many minor quantitative differences that appear to be inherited in a strictly Mendelian manner. EAST (1912) has reported multiple factor inheritance of the difference between typical *N. Bigelovii* and the variety known as *N. quadrivalvis.* All of the forms have 24 chromosomes as the haploid number.

The second group is the *alata* group, consisting of *N. alata, N. Forgetiana, N. Langsdorffii* and *N. Sanderae* — the horticultural species with variously colored flowers originating from the cross *N. Forgetiana* × *N. alata grandiflora.* The components of this group unquestionably should have specific rank on any morphological criteria; but in genetic behavior they are as one. Each type has 9 chromosomes (8 according to CHRISTOFF) as the haploid number, and will cross with any other type as readily as will the varieties of mankind. The hybrids are perfectly fertile and exhibit normal meiosis. *N. alata, N. Sanderae,* and presumably *N. Forgetiana* are self-sterile; *N. Langsdorffii* is self-fertile.

a. Inheritance of certain morphological characters in the *alata*-group

Hybrids between *N. Langsdorffii* and *N. alata* were studied by several of the early hybridizers including NAUDIN, GODRON, and FOCKE. They established several facts. Reciprocal hybrids were identical and in general intermediate in their characteristics, although the blue pollen of *N. Langsdorffii* was dominant. The F_1 luxuriant, uniform populations were perfectly fertile and produced variable F_2 populations.

EAST (1916) studied the inheritance of flower size in this hybrid. It was shown statistically that in corolla length the F_1 populations are as uniform as those produced by the parents after self-fertilization, while the F_2 populations are about 3 times as variable as the F_1 populations. *N. Langsdorffii* is reproduced in F_2 populations about once in 250 plants. These individuals, when selfed, produce populations in which the frequency distribution for corolla size is statistically identical with frequency distributions of pure *N. Langsdorffii.* In populations of nearly 600 F_2 plants the flower size of *N. alata* is approached but not equaled. The variabilities of F_3 families are invariably smaller than those of F_2 families. Apparently a small number of factors control the differences in corolla size; but the accumulation of factors for maximum size is hindered by some type of linkage phenomenon.

The behavior of two independent pairs of allelomorphs was also established. Blue pollen is dominant to yellow pollen, and yellow (chloroplast) is dominant to white.

An earlier study (EAST 1913) of inheritance of flower size, in a cross made between the small-flowered *N. Forgetiana* (perhaps a segregate of *N. Sanderae*) and the large-flowered *N. alata grandiflora*, yielded results not very different from the above. Reduplication of parental forms was such that only 4 factor differences need be postulated. There was a high degree of correlation (.61 ± .02) between length and spread of corolla in the F_2 generation. An error was promulgated in this paper, which should be corrected. The F_1 plants of this cross were thought to be self-fertile because selfings were made toward the end of the flowering season, and seed was obtained in great quantities. The plants are really self-sterile, as shown by early and mid-season pollinations. The end-season fertility exhibited has been called pseudo-fertility.

SACHS-SKALIŃSKA (1922) has shown the uniformity of the F_1 generation and the great variability of the F_2 generation, as to corolla size, in a cross between *N. Langsdorffii* and *N. Sanderae*. Both grandparental types were recovered.

In an earlier report SACHS-SKALIŃSKA (1921) presents an extended genetic analysis of flower color from the above cross. The anthocyan pigment in *N. Sanderae* is found to be controlled by 8 factors which act as follows:

F is a basic factor producing a violet color when acting alone.

C is a factor which produces no color alone, but acts in conjunction with F to produce red in the majority of the pigmented cells.

I_1, I_2 are factors conditioning intensity of color. Thus Fci_1i_2 is lilac, and FCi_1i_2 is rose. The presence of either I_1 or I_2 increases the intensity of color in either case. The presence of both I_1 and I_2 deepens the color to a still greater extent.

N determines that pigment shall appear on the exterior surface of the corolla.

T which acts only in the presence of N, extends this exterior pigmentation of the corolla to the tube.

P, R act only when F or F and C are present, and condition the distribution of pigment in the interior of the corolla. FCpr has only traces of red in the interior of the corolla. FCPr conditions pigment which does not extend very deeply into the parenchyma. FCPR forces the production of pigment more deeply in the parenchyma. R may act without P.

The author also sought to determine the action of the factors found in *N. Langsdorffii* which are concerned with the chlorophyll distribution which produce the various types of green. The matter does not seem to be very clear. Two factors Z_1 and Z_2 are first postulated. They are supposed to be rather loosely linked. Z_1z_2 corollas are dark green, z_1Z_2 corollas are less green approaching cream. Afterwards it is stated that Z_1 is in reality two factors, E causing green in the exterior of the corolla, and W in the presence of E causing green in the interior of the corolla face.

I suspect that one must be cautious in accepting SACHS-SKALIŃSKA's interpretations for this cross because of the self-sterility factors contributed by *N. Sanderae.* I endeavored to make similar analyses some years ago, but abandoned the task until the self-sterility matter was cleared up. How self-sterility affects the distribution of color factors will be explained in the next section of the paper.

b. Inheritance of self-sterility in the *alata*-group

Of the species in the *alata* group, *N. alata*, *N. Sanderae*, and presumably *N. Forgetiana* are self-sterile, as has already been noted; *N. Langsdorffii* is self-sterile. A study of hybrids between *N. Sanderae* and *N. alata grandiflora* (EAST and PARK 1917), all of which are self-sterile, uncovered the fact that each plant is not cross-fertile with every other plant, as DARWIN thought, but rather that there are groups of plants which are inter-sterile, though the individuals of each group are inter-fertile with the individuals of all other groups. The basis of this self- and cross-incompatiblity is a differential rate of pollen-tube growth. In an incompatible mating, the pollen-tubes grow slowly and the growth-curves approximate a straight line (EAST and

PARK 1918). In a compatible mating, the pollen-tubes grow rapidly, the growth-curves exhibiting rapid acceleration. The type of growth-curve is influenced by temperature and other external conditions, and by the physiological condition of the plant, as well as by hereditary factors. There are families in which the pollen-tube growth in incompatible matings is not enough slower than that of compatible matings to permit a satisfactory analysis of mating behavior, since at any time a few seeds are likely to be produced after an incompatible mating. On the other hand, there are families which are very satisfactory in this regard because compatible pollen tubes reach the ovaries in less than 3 days while incompatible pollen tubes are found less than half-way down the styles after 10 days, — this being ordinarily the maximum "life" of a flower. Even in most of these families, however, selfed seed can be obtained by keeping the plants at optimum temperature and pollinating the buds when the styles are half their normal length.

Since there is no block to fertilization in incompatible matings if the pollen tubes reach the ovary, advantage has been taken of the "pseudo-fertility" shown in bud pollinations to produce stocks by successive self pollinations. It was assumed that if self- and cross-incompatibility were controlled by hereditary factors that segregated and recombined in normal Mendelian fashion, such close inbreeding ought in a few generations to result in families where the factorial differences lying at the basis of self-sterility are reduced to a small number.

Numerous populations descending from the original *Sanderae—alata* hybrid by sib matings or "bud" selfings through several generations have now been tested and retested as to their mating reactions. In every family the plants have fallen into not more than four intra-sterile, interfertile classes. And their mating behavior has been such that the data can be interpreted by postulating a series of mutations of one chromosome locus, S_1, S_2, S_3. . . . This series of multiple allelomorphs now numbers 14, each distinct from all others.

The mating behavior easiest to analyze is that of families consisting of but two groups of plants, each plant within a group being sterile with all other members of that group but fertile with all plants of the second group. For this reason a number of two-class families characterized by various combinations of three classes which were termed

X, Y and Z were studied intensively. A class X female mated with a class Y male always gave an approximately equal number of plants belonging to classes Z and Y. A class Y female mated with a class X male, the reciprocal of the above mating, always gave an approximately equal number of plants belonging to classes X and Z. In other words, each type of cross produced two classes of intrasterile, interfertile plants in nearly equal numbers, and the class of the female was never represented.

These peculiar results may be interpreted (EAST and MANGELSDORF 1925, 1926; EAST 1926, 1927), if it be assumed that the active hereditary factors in these matings are S_1, S_2 and S_3, that class X is S_1S_3, class Y is S_1S_2, and class Z is S_2S_3, and that a plant affords a stimulus to pollen-tube growth *only* when the pollen grain bears a sterility factor other than its own.

When a Z female (S_2S_3) is crossed with a X male (S_1S_3) only the pollen bearing the factor S_1 is stimulated and functions. The resulting progeny consist therefore of the two classes S_1S_2 (class Y) and S_1S_3 (class X) in equal numbers. In the reciprocal cross, X female (S_1S_3) by Z male (S_2S_3), the mother plant (S_1S_3) again affords stimulus only to gametes bearing factors other than its own, — this time the S_2 pollen. The result is the production of the two classes Y (S_1S_2) and Z (S_2S_3) in equal numbers.

Practically all possible tests of this hypothesis have now been made and it has been substantiated in every case. By selfing young buds of the heterozygous classes, X, Y and Z, it has been shown that ordinary Mendelian segregation occurs, and that the anomalous results usually obtained come about only because ordinarily the opportunity for union of like gametes is lacking. When a heterozygous class such as class Y (S_1S_2) is selfed in the very young bud, however, equal opportunity is furnished for all possible combinations of gametes. The resulting progeny consist of plants having approximately the ratio 1 S_1S_1 plus 2 S_1S_2 plus 1 S_2S_2, and these classes show the sterility reactions expected of them. For example, S_1S_1 plants crossed with S_2S_2 plants give only plants of the one class S_1S_2 no matter which way the cross is made. These F_1 plants are sterile with both the parental types, S_1S_1 and S_2S_2, when those types are used as males, because neither S_1 pollen nor S_2 pollen functions normally on S_1S_2 plants. S_1S_2 plants will cross with either parent when used as males, on the other hand, because S_2 pollen functions on the S_1S_1 parent and S_1 pollen functions on the S_2S_2 parent. The result in each case is the production of S_1S_2 plants only.

An extraordinary confirmation of the hypothesis has come from the work of BRIEGER and MANGELSDORF (1926), which has shown that allelomorphic series, $S_1 \ldots S_{14}$ (tests made on three allelomorphs), is at a locus on the same chromosome as a factorial pair C and c. Crossing-over is in the neighborhood of 18 per cent in both sexes. This allelomorphic pair is such that CC or Cc plants show a purplish color at the base of the stem, color in the flowers, and brown, rounded seeds; cc plants show a green stem, white flowers, and white angular seeds.

It is clear that self-incompatibility, and its corollary cross-incompatibility, is controlled by a limited number of hereditary factors whose function is to produce differential pollen-tube growth. Self-sterile plants are not specialized individually in this respect, as DARWIN and JOST assumed; but are specialized in groups. The fourteen allelomorphic factors thus far isolated form a group larger than any other known group of multiple allelomorphs and indicate an odd or peculiar situation not usually obtaining in genetic work; but even if the series should be materially extended it is easier to accept than an hypothesis postulating individual specialization. Whether more than one chromosome locus is effective in controlling the behavior of self-sterile plants is unknown. It is a matter of great importance for the physiological interpretation of the phenomenon and is being investigated.

In 1919, EAST reported results from crosses between *N. Langsdorffii* and *N. Sanderae*, and concluded that the relation between self-fertility and self-sterility is mono-factorial with dominance of self-sterility. Recent work in which plants bearing known self-sterility factors have been crossed with *N. Langsdorffii* has made these conclusions doubtful.

INTRA-SPECIFIC HYBRIDS IN NICOTIANA

Nicotiana Tabacum L.

The commercial importance of *Nicotiana Tabacum* has given rise to a huge literature on tobacco breeding. The principal contributing countries have been Germany, Holland, India, Italy, and the United States. The more important papers are cited in the bibliography, but it does not seem desirable to discuss them here.

Nicotiana Tabacum exhibits extensive variation particularly in color and size of flower, in shape and texture of leaves, and in height and number of leaves. These variations have been considered in detail by COMES (1899, 1905), who refers all existing forms to six varieties; viz., *fruticosa* HOOK, *lancifolia* (W.) COMES, *virginica* (AGDH.) COMES, *brasiliensis* COMES, *havanensis* (LAG.) COMES, and *macrophylla* SCHRANK. The classification is based largely upon leaf-shape, though corolla-shape plays a part. *Virginica* and *brasiliensis* are hypothetical varieties not now found in a pure state.

ANASTASIA (1907) has criticized this classification. He would have the number of basic varieties reduced to four, — *havanensis, brasiliensis, virginica,* and *purpurea*. To this criticism COMES (1908) has replied.

The HOWARDS (1910) have also attempted to classify the cultivated forms of *N. Tabacum,* using the Indian types. They recognized fifty-one kinds, and keyed them on a basis of leaf-form and length of internode. The work of these authors represents perhaps the first attempt to support a morphological classification of tobaccos by genetic experiments. They made a large series of crosses, and studied the recombination forms intensively. Segregate types were found (G. L. C. HOWARD 1913) which exceeded the limits of the parents in numerous cases. The favorite key character was not exempt, for "petiolate" forms were obtained by crossing two "sessile" forms. Such results showed the futility of trying to trace the derivation of a variable cultivated species by a purely morphological classification (Cf. SETCHELL, GOODSPEED, and CLAUSEN 1922). Miss HOWARD in fact, came to the conclusion that her scheme of classification was merely a useful empirical key without phylogenetic value; and with this conclusion most geneticists will agree.

In the nineteenth century literature on tobacco breeding, the idea was persistent that a change in soil and climatic conditions was the direct cause of tobacco variability. It was found, for example, that seed harvested from selected plants in Cuba or Sumatra, when brought to the United States produced all sorts of diverse forms of plants. When the United States Department of Agriculture became interested in tobacco breeding and instituted a large series of experiments, the most important and emphatic conclusion promulgated, was that change from one environment to another caused a "breaking up of the type" (See especially SHAMEL 1904, 1910; SHAMEL and COBEY 1906, 1907).

The careful experiments of HASSELBRING (1912), HAYES (1912, 1913), EAST and HAYES (1912, 1914), HAYES, EAST and BEINHART (1913), HONING (1915, 1916, 1918, 1919) and DAVID (1925) have shown that this idea has no foundation in fact. Though *N. Tabacum* will stand continued self-fertilization without deterioration (EAST and HAYES 1912), and might be called a naturally self-fertilized plant because of the frequency with which selfing occurs, nevertheless, cross-pollination is sufficiently common that selfed seeds are likely to show numerous recombination types. The socalled "breaking up of type", therefore, is a result of normal inheritance and not a Lamarckian effect. It is of considerable interest to note in this connection the difference to be expected when dealing with selected seed of *N. Tabacum* and with the wind-pollinated plant *Zea mays*. Maize is as variable as tobacco. But it is so constantly crossed and recrossed in every possible combination, that even the plants produced from a single individual without control of the pollen, show a spurious uniformity. Only when a plant is artificially selfed does the resulting progeny show the true variability to be expected from the recombination of the factorial differences involved. *N. Tabacum,* on the other hand, exhibits enough natural self-fertilization to give results comparable to those obtained after selfing a maize plant.

A study of any one of the papers cited above brings out the tremendous amount of variability in this species. A very large number of genes have varied, and this variability makes it possible to produce by crossing and selection a type with leaves of almost any number, size, shape, and texture. The development of the "Round Tip" tobacco (EAST and JONES 1921), by crossing the varieties "Broadleaf" and "Sumatra", is a case in point. The characteristics of an idealistic plant required for cigar wrappers were listed beforehand. The parent plants were selected from a knowledge of their genetic characteristics. And the hypothetical variety was actually produced. Perhaps the best illustration of the variability which tobacco shows in quantitative characters, however, is shown by a selection experiment on number of leaves in a cross "Havana × Sumatra". A series of selections from F_1 to F_8 were reported in 1914 (EAST and HAYES). The experiment was designed to throw some light on the genetic meaning of selection and was carried on to generation F_{12}; but since biological ideas regarding the true significance of selection had by that time become rather concordant, a full account of the experiment was not published. In brief, it may be stated that selection in self-fertilized lines for a high number and a low number of leaves showed that any desired average number from about 12 to 30 could be obtained combined with various types of shape, quality, etc. The lowest coefficient of variability obtained was about 4.00 per cent, and this coefficient may be considered to measure a residuum of fluctuating variability in a genetically homozygous strain. It may be obtained in the F_2 generation, of course, when the experiment is carried on in a sufficiently extensive scale; but in practice it sometimes takes 12 years or more before no further change is obtained by selection. (See also JENSEN 1916, JOCHEMS 1921).

An interesting feature of tobacco variability concerns the nicotine content. GARNER (1909) has determined that either high-nicotine or low-nicotine strains can be produced by selecting the proper genotypes.

In spite of the extraordinary variability of tobacco, few mutations have been observed under controlled conditions. Some of the earlier post-mendelian studies, however, are concerned with teratological characters of reasonably well-known origin. LODEWIJKS (1911) investigated several types. A double flower was recessive to the normal. An *aurea* condition was thought to be a heterozygous type. One family of *aurea*, when used as the female in crosses with green, gave 83 per cent *aurea* progeny, while the reciprocal gave 48 per cent *aurea*. Another family of *aurea* used as the female in crosses with green gave 48 per cent *aurea* progeny, while the reciprocal gave 43 per cent. An indeterminate or giant form crossed with normal gave variable results. LODEWIJKS' results may be compared with those of JONES (1921). The variability is evidently due to external conditions. Tricotyly, fasciation, and certain quantitative characters in leaves were also investigated and shown to be conditioned by a large number of factors. WHITE (1913, 1914, 1916) has studied in great detail a fasciated mutant which came from selfed seed of the "Cuban" variety, and appeared at Alquiza in Cuba in 1907. This mutation affected every part of the plant occasionally, including even the maturation divisions, though apparently only gametes with 24 chromosomes were functional. Crossed with normal tobaccos this mutant showed imperfect dominance and behaved as a single genetic factor. But selection experiments indicated that numerous subsidiary factors affected its expression. WHITE also found that a calycanthemous mutant of unknown origin exhibited partial dominance and behaved as a monohybrid difference in crosses with normal plants.

A frequently recurring mutation in various varieties of tobacco is that known as "gigantism", or "indeterminate growth". The plants characterized by this feature continue to grow, under ordinary conditions, without producing the usual terminal inflorescence. GARNER and ALLARD (1920) have shown, however, that the blossoming of such plants is conditioned by the relative length of night and day. When the day is artificially shortened, the "indeterminate" plants produce flowers.

Indeterminate mutations have been reported by HUNGER (1905), LODEWIJKS (1911), and HONING (1914) in the Deli tobacco of Sumatra and Java; by GARNER (1912) in Maryland Mammoth; by HAYES and BEINHART (1914) and HAYES (1915) in Cuban and Connecticut Havana; and by ALLARD (1919) in several other varieties. HAYES and BEINHART (1914) observed 5 such mutations, and estimated the mutation frequency to be 1 per 1,000,000. Since the plants bred true to their characteristics, it was assumed that mutation occurred after fertilization. It is therefore comparable to a bud-mutation (EAST 1917, ALLARD 1919). LODEWIJKS (1911) observed the occurrence of "indeterminate" types which bred true and also of intermediate types which threw 25 per cent "indeterminate" plants. HONING's experience (1914) was somewhat similar, though he thought that several distinct types existed. And he even found that normal appearing plants occasionally produced 100 per cent "indeterminate" plants. ALLARD (1919) corroborated the Dutch observations of various types of indeterminates, these being found even in the progeny of a single plant. But generally speaking, the normal condition was dominant in crosses with other tobacco varieties and with the species *N. sylvestris* and *N. glutinosa*. In several intraspecific crosses where 1820 F_2 plants were grown, 439 plants had the indeterminate character.

JONES (1921) reported a cross between an indeterminate Cuban plant and the normal type of the same variety. The normal form was dominant. In the F_2 generation, when the seeds were sown in flats and the best seedlings transplanted, the ratio was 82 normal to 1 abnormal. When a method more suitable for obtaining a random sample of the F_2 generation was used, the ratio was 7.4 to 1. By studying the frequency distribution of the leaf number, however, JONES was able to show that in all probability the true ratio was in the neighborhood of 3 to 1. The character, therefore, is one of the type which can be changed materially by altering the conditions during development.

Two haploid tobaccos have been described by CLAUSEN and MANN (1924). Both appeared in crosses between varieties of *N. Tabacum* with *N. sylvestris*, and were termed the *purpurea* and the *macrophylla* haploids after the maternal varieties from which they originated. They were reduced replicas of the types from which they came, thus corroborating what BLAKESLEE had found in *Datura* and GAINES and AASE in wheat. In the first report, the pollen was stated to be wholly defective, abortive reduction being a haphazard process with irregular chromosome distribution; but the plants were kept alive by asexual reproduction. In a second paper, (CHIPMAN and GOODSPEED 1927) giving more complete cytological details for *purpurea* haploid, some viable pollen having the full haploid chromosome set is stated to have been produced. The embryo sacs, on the other hand, fail to mature. Several hybrid plants have been obtained by crossing *purpurea*-diploid by *purpurea* and by *macrophylla* haploids.

Meiosis in the *purpurea* haploid exhibits numerous peculiarities. The prophases show a normal synizesis, a second contraction, a diakenesis, etc.; but these stages show as a mixture in the anther sac, rather than an orderly succession of phases. In later stages, while there is no uniformity, it appears that there is a true metaphase with the chromosomes coming to lie along the axis of a bipolar spindle in such a manner as to allow random distribution of the twenty-four univalents which it holds. In other cases, disturbance is so great that bipolarity is not attained until after the normal period for chromosome distribution. The chromosomes are then held in peaks of the multipolar spindle which becomes in part bipolar. In most cases, 24 chromosomes can be counted from the supposed first metaphase to anaphase. Exceptionally, bivalents appear, corresponding in size to diploid bivalents. But the authors believe that this is not a reflection of pairing before third contraction, because no evidence of pairing has been obtained at diakenesis or earlier. In 80 pollen mother cells, 48 showed no dividing univalents; in varying frequency from 1 to 8 divided; and in 4 cases 24 divided. The successful division of all 24 chromosomes presumably accounts for the formation of dyads and the production of viable pollen grains. But the evidence is that the heterotypic division is not replaced by a homotypic division as in *Datura*; rather is it suspended.

Lately Miss RUTTLE (1928) has described and figured three additional haploids of *N. Tabacum purpurea* and two additional haploids of *N. Tabacum* "Cuba". The somatic chromosome complex of these plants has been studied and compared with the somatic chromosome complex of diploid *N. Tabacum.* The diploid plants' cells contain 48 chromosomes, some of which bear small proximal satellites (less than four). Among 82 root tips from the haploid plants that were examined, 52 were diploid, 22 haploid, and 8 a mixture of haploid and diploid cells. In cases of the last type, the diploid area varied from a few isolated cells in the neighborhood of the growing point to large sectors extending through the whole length of the meristematic tissues. This aberrant diploidy appears to be limited to the root tips, for it was not found in archesporial tissue, in pollen mother cells, or in ovules.

The botanical department at the University of California has made an extensive study of intraspecific hybrids of *N. Tabacum.* The inception of this work was SETCHELL's endeavor (1912) to classify the stem types of *N. Tabacum.* In 1921 and 1922, SETCHELL, GOODSPEED and CLAUSEN reported on a series of crosses which may be called *angustifolia—macrophylla, calycina—virginica,* and *alba—macrophylla.*

The outstanding result of the investigation, as in the case of the similar investigations carried out at the Connecticut Agricultural station by EAST, HAYES, and JONES, was to demonstrate the complexity of the genetic differences in *Nicotiana Tabacum* and the validity of the multiple factor hypothesis as a description of the heredity of quantitative characters. In addition, the behavior of a pair of genes, Rr, conditioning flower color, was established. Pink is partially dominant over red, but the three types RR, Rr, and rr can be distinguished fairly easily. Similar results had already been obtained by ALLARD (1919). Leaf base was also analyzed. Three pairs of factors are involved. SS is long petioled like *angustifolia*; ss is broadly sessile like *macrophylla*. LL with SS is long petioled; ll with SS is short petioled like *latifolia*. AA with ss is sessile, aa with ss is auriculate. KELANEY (1925), however, is of the opinion that genotypes SSLLAA, SsLLAA, and SSLlAA are not petioled but lanceolate.

The result of the *calycina—virginica* cross was to show that in the allelomorphic pair Cc (calycine), the normal form of flower was dominant over the hose-in-hose type. WHITE (1916) working with the same characters did not get this result. In his crosses, the hose-in-hose type was partially dominant over the normal.

In the *alba—macrophylla* crosses, the data are interpreted as the expression of Color (W) and no color (w). *Alba* is RRww, *macrophylla* is rrWW.

CHRISTOFF (1925*a*) suggests that three factors condition flower color. The red color is determined by the factor W, and the light rose color by S which in the presence of R acts as a diluter on W and in the absence of R neutralizes W. Thus WrS and wrS are both light rose, WRS is rose, Wrs is red, and wRs and wRS are white. CHRISTOFF also suggests simple Mendelian factors for corolla form and size, but these suggestions can hardly be used to interpret such complex data.

In a later paper on flower color (GOODSPEED and CLAUSEN 1916) the following formulae are suggested: WWRRPP carmine, WWRRpp light pink, WWrrpp red, and wwRRpp white. KELANEY (1925) distinguishes carmine from red by its filament color.

CLAUSEN and GOODSPEED (1922) described two natural periclinal chimeras in *N. Tabacum* hybrids. In the F_1 of a cross between red-flowered *macrophylla* and white-flowered *Cuba*, a plant otherwise pink-flowered produced white flowers on one branch. Plants raised from selfed seed produced by each type of branch gave practically identical ratios of pink, red, and white flowers. The other chimera arose in a carmine-flowered F_1 of *purpurea* × *Cuba*. The varying branch had pink flowers. Both types selfed gave essentially similar results. It was therefore assumed that the variations had not affected the hypodermal layers which produce the gametes. To test the chimera hypothesis, carmine and pink clones were propagated both by stem cuttings and by root cuttings. The stem cuttings perpetuated the pink and the carmine flowers respectively. The root cuttings of both gave plants producing carmine flowers, "which is in agreement with the assumption that the pink bud variant consists of a central cylinder of carmine of normal genetic constitution enclosed in a dermatogen which is genetically pink."

In addition to the observations already noted, KELANEY (1925) has found that triple allelomorphs C—c—C^a condition certain flower shapes. Ap and ap condition normal and apetalous types respectively but effective only in conjunction with C^a when it gives a petaloid calyx termed the KLEBS' form. C therefore is normal, c is calycine, and c^a with Ap is KLEBS' type.

More recently HONING (1927) has investigated the inheritance of a dwarf form which he found to be dominant in crosses with Deli tobacco. He also suggests a factor F conditioning the winged leaf base of Hatano tobacco as distinguished from the ordinary sessile leaf of Deli. A deformis factor n is epistatic to FF, Ff, and ff. All nn types are petioled.

ARISZ (1927) has studied catacorolla and other teratological corolla forms as well as certain tissue proliferations from the midrid of the leaf. The inheritance is complex and has not been satisfactorily analyzed.

Besides these types of inheritance, CLAUSEN and GOODSPEED (1923, 1926) have investigated two characters which are known to be determined by chromosomes differences after the manner of certain characters in *Drosophila* and *Datura*. These are the trisomic ($2n + 1$) character "enlarged", and the monosomic ($2n - 1$) character "fluted'

The character "enlarged", depending upon an extra chromosome, is transmitted by about 35.5 per cent of the functional ovules and by about 3.4 per cent of the functional pollen grains of an "enlarged" plant. The tetrasomic condition is obtained and is called super-enlarged. Super-enlarged selfed gives on the average 20 per cent super-enlarged, 77 per cent enlarged, and 3 per cent normal. About 94 per cent of the ovules and about 52 per cent of the pollen grains transmit the enlarged character, which is incompatible with the idea that permanent increase in chromosome number may result from non-disjunction.

KELANEY (1925) found that broad (A) and auriculate (a) leaves, and carmine (P) and pink (p) flowers are allelomorphs of the same linkage group with 7.5 per cent crossing over. By appropriate tests he discovered that "enlarged" belongs to the same group.

The monosomic (2n — 1) form "fluted" occurs spontaneously in certain *N. Tabacum* cultures with a frequency of about 1 : 150. It decreases the flower size, in contrast to the effect of enlarged; but it does not involve the same chromosome set. The character is transmitted by about 60 per cent of the functional ovules and by about 2 per cent of the functional pollen grains. Crosses between fluted and the enlarged pink-auriculate group gave the information that this chromosome set was not involved. Five other factors were tested with fluted; but it exhibited no linkage with them.

Recently Goodspeed and Olson (1927) report great acceleration of mutation rates in *N. Tabacum* by the use of X rays. Plant buds on the terminal inflorescence were rayed for 10 minutes on one plant and for 20 minutes on a second plant at a distance of approximately 30 cm., with a Coolidge X ray tube operated by a transformer without rectification at an effective potential of 50.000 volts and a current of 2.8 milliamperes. The variety used was *purpurea*.

Over 1000 plants from the first X raying have now been grown to maturity. They came from seven self-pollinated capsules of X rayed buds. Variant plants made up over one-half of the populations. In one population, which was studied intensively, 136 out of 168 plants were variants. In some cases, the variation was largely a question of reduced fertility, but in most cases morphological changes were observable. No two variants were alike. The habit of growth was dwarf, low or tall; the leaves were large, small, lanceolate, cordate, smooth, wavy, corrugated, dark green, grayish green, petiolate, ruffled, and decurrent; the flowers were small to large, fluted, calycine, folded, notched, 10-sided, light pink, pink, red, and reddish purple; the ovaries were 2-, 3-, and 4-celled.

About half of 15 plants studied cytologically exhibited no departure from the normal. The remainder showed cytological abnormalities of all kinds, both in somatic and meiotic divisions. Of six variants studied in detail, three showed a chromosome with a satellite, a condition not found normally at meiosis, though common in somatic mitoses. One plant had only 21 pairs of chromosomes.

Plants from seed of X rayed flowers of *N. Bigelovii Wallacei* and of

N. rustica pumila were also grown in smaller numbers. Apparently the treatment had no effect upon *N. Bigelovii*; but variants were found in the progeny of the *N. rustica* plants.

Treated buds of *N. suaveolens* and *N. nudicaulis* were killed.

In connection with our discussion of *N. Tabacum*, it may be pointed out that occasional apogamous reproduction in a Cuban variety was established by Mrs. HAIG-THOMAS (1909, 1913). This observation was confirmed by BATESON (see discussion of the 1913 paper at the Fourth International Conference) and by GOODSPEED (1915) in plants grown from seed furnished by Mrs. HAIG-THOMAS. HOWARD and RAM (1924) noted parthenocarpy but no apogamy or parthenogenesis in any variety, not even in the Cuban variety used by Mrs. HAIG-THOMAS which she had kindly sent them. GOODSPEED found no apogamy in other varieties, though parthenocarpic fruits were produced in great numbers. EAST (see WELLINGTON 1913) also has been unable to discover apogamy either in *N. Tabacum* or in some sixteen other species. But seed was obtained which produced normal maternal plants and also true hybrids from crosses of *N. paniculata* × *N. alata grandiflora*, *N. rustica* × *N. Tabacum*, and *N. Tabacum* × *N. Bigelovii*; seed which produced maternal plants and no true hybrids from crosses *N. paniculata* × *N. longiflora*, *N. paniculata* × *N. Sanderae*, and *N. Bigelovii* × *N. sylvestris*; and seed which produced maternals and occasional hybrids from cross *N. Tabacum lancifolia* × *N. alata grandiflora*. In these cases of induced apogamy, it is unknown whether adventitious embryos were produced from the somatic tissue of the nucellus or from haploid cells which later doubled. In several plants investigated the number of chromosomes was diploid. In view of the extraordinarily high number of maternal plants produced, it seems more probable that there was induced parthenogenesis with a doubling of the chromosomes. WELLINGTON (1913) was unable to induce parthenogenesis or apogamy in sixteen species of *Nicotiana* by mutilation or by chemical agents.

Nicotiana rustica L.

Nicotiana rustica is second in commercial importance only to *N. Tabacum*. COMES (1899) refers existing forms to six stem types on a basis of leaf shape, internode length, corolla-like shape, and capsule shape. Variety *texana* COMES, supposed to be a native of Mexico, is said to have been the first variety introduced into Portugal. Varieties *asiatica* SCHRANK. and *brasilia* SCHRANK. are the varieties principally grown for snuff in Asia and Europe respectively. Variety *iamaciensis* COMES and *scabra* COMES are wild types from Central America and the West Indies, and from South America respectively. Variety *humilis* SCHRANK. is a small form grown for snuff.

The HOWARDS (1910) grew some 20 forms and keyed them on a basis of long and short internodes, the plants with short internodes being divided into loose-flowered, crowded flowered, and compact-flowered inflorescences.

The only simple qualitative characters, the inheritance of which has been determined, are a white stemmed chlorotic *aurea* type which is recessive to the normal type (ALLARD 1919), undulate leaf margin allelomorphic but not dominant to flat leaf margin (HOWARD 1924), and brown seed dominant to white seed (EAST unpublished).

Miss HOWARD (1924) made a number of crosses between various types of *N. rustica*, and followed numerous characters. There was the usual increase of variability and other phenomena characteristic of multiple-factor inheritance. The present writer has also made numerous similar crosses, but since the only result obtained was added evidence of multiple-factor inheritance, publication was thought inadvisable.

Miss HOWARD (1924) discovered no apogamy, parthenogenesis, or parthenocarpy in *N. rustica*. All varieties except those in which the pistil is longer than the stamens were found to self-fertilize naturally under ordinary circumstances.

N. longiflora CAV.

EAST (1916*b*) has reported a study of the inheritance of corolla length between long-flowered and short-flowered varieties of *N. longiflora*. It is given extended notice here because in this paper the mathematical requirements for a Mendelian analysis of the inheritance of quantitative characters were set forth for the first time. For other accounts of the inheritance of quantitative characters in *Nicotiana*, one may consult the following papers: EAST, 1913, 1916, 1916*a*, 1921; EAST and HAYES, 1914; FRIMMEL, 1924; GOODSPEED, 1912, 1913*b*, 1915*c*; GOODSPEED and CLAUSEN, 1915, 1915*a*; HAYES, 1912, 1913, 1913*a*; HAYES, EAST and BEINHART, 1913; HONING, 1920*a*;

HAUSER, 1911; JENSEN, 1916; LODEWIJKS, 1911; SETCHELL, GOODSPEED and CLAUSEN, 1922.

In the cross under consideration, No. 383, used as the female parent, was a small variety of *N. longiflora* obtained from Professor A. SPLENDORE under the name *N. plumbaginifolia* VIV. Descendants of this plant were used by CHRISTOFF (1928) in determining a chromosome number for *N. plumbaginifolia*; but the plants are not specifically distinct from *N. longiflora*. No. 330, the variety used as the male parent was typical of *N. longiflora*. It was obtained from Professor W. A. SETCHELL.

Inheritance of flower size is of special interest, for EAST (1916*a*) has shown that flowers of various species of *Nicotiana* are scarcely influenced at all by external conditions. Although, as GOODSPEED and CLAUSEN (1915, 1915*a*) maintain, there is usually a slight decrease in flower size toward the end of the flowering season, if one measures flowers from similarly located branches and at similar places on the inflorescence, single measurements represent the plant phenotype to within a millimeter. The same distribution of flower size is exhibited when parts of a given population are starved in small pots or when they are given the best of conditions in the open field. The measurements given in the following tables, therefore, give the phenotypes of the plants considered.

Studies of Mendelian phenomena in cases involving simple qualitative character permit one to state eight requirements, apparently independent mathematically, which should be met by the pedigree-culture data on size characters when all populations succeeding the original cross are obtained by self fertilization.

1. Crosses between individuals belonging to races which from long-continued self-fertilization or other close inbreeding approach a homozygous condition, should give F_1 populations comparable to the parental races in uniformity.

2. In all cases where the parent individuals may reasonably be presumed to approach complete homozygosis, F_2 frequency distributions arising from extreme variants of the F_1 population, should be practically identical, since in this case all F_1 variation should be due to external conditions.

3. The variability of the F_2 population from such crosses should be much greater than that of the F_1 population.

4. When a sufficient number of F_2 individuals are available, the grandparental types should be recovered.

5. In certain cases individuals should be produced in F_2 that show a more extreme deviation than is found in the frequency distribution of either grandparent. This phenomenon was predicted by the writer (EAST, 1910) as an expected result of Mendelian recombination before actual cases had been discovered.

6. Individuals from various points on the frequency curve of an F_2 population, should give F_3 populations differing markedly in their modes and means.

7. Individuals either from the same or from different points on the frequency curve of an F_2 population should give F_3 populations of diverse variabilities extending from that of the original parents to that of the F_2 generation.

8. In generations succeeding the F_2, the variability of any family may be less but never greater than the variability of the population from which it came.

Table III shows the frequency distribution for corolla length for the relatively homozygous parents for the F_1 generation, for two F_2 generations, and for various populations raised in succeeding generations. Table IV shows certain statistical constants for these frequency distributions.

It is obvious that all of these mathematical requirements are met except that there does not always appear to be a continuous reduction in the coefficient of variation due to the expected trend toward homozygosis produced by successive self-fertilizations. But the few cases where a later population has a higher coefficient of variation than an ancestral population are within the limits of expectancy on the theory of probability. The F_1 generation is as constant as that of the more variable parent. The variability of the F_2 generations greatly exceeds that of the F_1 generation. The frequency distributions of the two F_2 generations are similar in type. The F_3 and later generations accord with the theoretical expectancy of Mendelian but not of Galtonian inheritance. In thirteen out of fifteen fraternities descended from the two F_2 populations, there was regression toward the mean of the fraternity from which the parent came; but two individuals produced populations with means further removed from the means of the parental populations than were the parent individuals themselves.

TABLE III. — FREQUENCY DISTRIBUTIONS FOR COROLLA LENGTH IN A CROSS BETWEEN VARIETIES OF NICOTIANA LONGIFLORA CAV.

Designation	Year	Generation	Parent size	Class centers in millimeters																						
				34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100
No. 383	1911	—	—	—	13	80	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 383	1912	—	—	1	4	28	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 383	1913	—	—	—	4	32	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 330	1911	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	22	49	11	—
No. 330	1912	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	16	32	6	1
No. 330	1913	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	7	10	2	—
No. 383 × 330	1911	F$_1$	—	—	—	—	—	—	—	—	4	10	41	75	40	3	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 1	1912	F$_2$	61	—	—	—	—	—	—	1	5	16	23	18	62	37	25	16	4	2	2	—	—	—	—	—
No. (383 × 330) 2	1912	F$_2$	61	—	—	—	—	—	—	2	4	2	24	37	31	38	35	27	21	5	6	1	—	—	—	—
No. (383 × 330) 1–1	1913	F$_3$	72	—	—	—	—	—	—	—	—	—	4	20	25	59	41	19	2	—	—	—	—	—	—	—
No. (383 × 330) 1–2	1913	F$_3$	46	—	—	—	1	4	26	44	38	22	7	1	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 1–3	1913	F$_3$	50	—	—	—	6	20	53	49	15	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 1–4	1913	F$_3$	60	—	—	—	2	3	9	25	37	70	19	10	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 2–1	1913	F$_3$	77	—	—	—	—	—	1	0	1	1	1	2	16	33	43	34	20	6	1	—	—	—	—	—
No. (383 × 330) 2–3	1913	F$_3$	81	—	—	—	—	—	—	—	—	—	1	1	8	16	20	32	41	17	3	3	1	—	—	—
No. (383 × 330) 2–4	1913	F$_3$	80	—	—	—	—	—	—	—	—	—	2	8	14	21	39	39	32	10	1	—	—	—	—	—
No. (383 × 330) 2–5	1913	F$_3$	50	—	—	—	—	7	25	55	55	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 2–6	1913	F$_3$	82	—	—	—	—	—	—	—	—	—	—	—	3	5	12	20	40	41	30	9	2	—	—	—
No. (383 × 330) 1–2–1	1914	F$_4$	44	—	—	8	42	95	38	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 1–3–1	1914	F$_4$	43	—	—	2	23	122	41	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 2–6–1	1914	F$_4$	85	—	—	—	—	—	—	—	—	—	—	—	—	—	4	9	38	75	59	6	3	1	—	—
No. (383 × 330) 2–6–2	1914	F$_4$	87	—	—	—	—	—	—	—	—	—	—	—	4	5	6	11	21	33	41	29	8	5	1	—
No. (383 × 330) 1–3–1–1	1915	F$_5$	41	3	6	48	90	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. (383 × 330) 2–6–2–1	1915	F$_5$	90	—	—	—	—	—	—	—	—	—	—	—	—	—	2	3	8	14	20	25	25	20	8	—

TABLE IV. — STATISTICAL CONSTANTS OF THE FREQUENCY DISTRIBUTIONS SHOWN IN TABLE III

Designation	No. Ind.	Mean	S. D. in mm	C. V. in percent
No. 383—1911	125	40.46 ± .11	1.75 ± .07	4.33 ± .18
No. 383—1912	49	40.61 ± .19	2.00 ± .14	4.92 ± .34
No. 383—1913	37	39.76 ± .12	1.09 ± .09	2.74 ± .21
No. 330—1911	88	93.22 ± .16	2.29 ± .12	2.46 ± .13
No. 330—1912	57	93.37 ± .20	2.23 ± .14	2.39 ± .15
No. 330—1913	24	92.12 ± .37	2.70 ± .26	2.93 ± .30
No. (330 × 383) F_1	173	63.53 ± .15	2.92 ± .11	4.60 ± .17
No. (330 × 383) –1 F_2	211	67.51 ± .27	5.91 ± .19	8.75 ± .29
No. (330 × 383) –2 F_2	233	69.78 ± .30	6.79 ± .21	9.73 ± .30
No. (330 × 383) 1–1 F_3	170	73.14 ± .20	3.82 ± .14	5.22 ± .19
No. (330 × 383) 1–2 F_3	143	53.47 ± .21	3.74 ± .15	6.99 ± .28
No. (330 × 383) 1–3 F_3	147	50.20 ± .18	3.17 ± .12	6.31 ± .25
No. (330 × 383) 1–4 F_3	175	56.34 ± .21	4.07 ± .15	7.22 ± .26
No. (330 × 383) 2–1 F_3	159	73.04 ± .27	5.00 ± .19	6.85 ± .26
No. (330 × 383) 2–3 F_3	143	76.34 ± .29	5.06 ± .20	6.63 ± .26
No. (330 × 383) 2–4 F_3	166	74.01 ± .25	4.85 ± .18	6.55 ± .24
No. (330 × 383) 2–5 F_3	160	52.97 ± .16	3.04 ± .11	5.74 ± .22
No. (330 × 383) 2–6 F_3	162	80.20 ± .25	4.76 ± .18	5.93 ± .22
No. (33) × 383) 1–2–1 F_4	184	45.71 ± .12	2.37 ± .08	5.18 ± .18
No. (330 × 383) 1–3–1 F_4	189	46.25 ± .09	1.87 ± .06	4.04 ± .14
No. (330 × 383) 2–6–1 F_4	195	82.25 ± .16	3.30 ± .11	4.01 ± .14
No. (330 × 383) 2–6–2 F_4	164	82.86 ± .31	5.83 ± .22	7.04 ± .26
No. (330 × 383) 1–3–1–1 F_5	161	41.98 ± .12	2.30 ± .09	5.49 ± .21
No. (330 × 383) 2–6–2–1 F_5	125	87.88 ± .33	5.52 ± .24	6.28 ± .27

Nicotiana acuminata GRAH.

GOODSPEED (1912, 1913, 1915) has made an extensive study of the inheritance of corolla diameter on three varities of *N. acuminata*. The corolla diameter of these varieties was 13 mm., 20 mm., and 27 mm., respectively, — fluctuation from the average never exceeding plus or minus 2 mm. From crosses between these varieties, including reciprocals, five populations were grown. Each group produced plants in which the average diameters of the corollas were approximately the mathematical average of the parents. Fluctuation was from 13 to 30 mm. In the F_2 generations, certain populations apparently exhibited narrow variability and others a variability much transcending the F_1 generation; but the number of plants raised (15 to 18) was too small to draw definite conclusions on this score. In the final report (1915), however, both the F_2 and the F_3 generations showed the variability to be expected if flower diameter is governed by several genes.

RELATIONSHIPS OF NICOTIANA SPECIES

Investigations on the genus *Nicotiana* have resulted in several extensions of genetic theory; they have not enabled us, thus far, to interpret the relationships between the species as clearly as we had hoped. Nevertheless, an approach to a true and intimate knowledge of the biology of the genus has been made.

Much remains to be learned regarding the physiology of the gametophytes during the period preceding actual gametic union. There it indication, for example, that hybridization takes place somewhat more easily in cases where there is a difference in chromosome number, when the species having the higher chromosome number is used as the female (CHRISTOFF 1928); but there are notable exceptions to this rule. It has also been found (KOSTOFF) that parthenocarpy occurs in incompatible matings only if the generative nucleus of the species used as the male reaches and enters the microphyte. But such pieces of information are small oases in a desert of ignorance.

Again, there is little clear-cut information regarding the reasons why the growth of embryo and of endosperm stops at various stages in development. Both endosperm formation and embryo growth may stop at almost any stage. Apparently perfect seeds may be formed which will not germinate. Germination may be very low, and yet the seedlings that are produced will develop into vigorous plants. Germination may be high, and the seedlings will die after having produced one or two leaves. Some hybrids are invariably small and weak; others are extremely vigorous. There is no correlation between somatic vigor and the formation of viable gametes. The reasons for such phenomena await further investigation.

On the other hand, it seems fair to say that, even though no very concrete conclusions can be drawn as to the actual origin of *Nicotiana* species, certain justifiable inferences can be made as to genetic compatibility which aid materially in understanding the genus as a whole. These relationships are shown graphically in Figures 1 and 2 (see p. 257 a. 259). *N. Tabacum* is clearly the type of a section of the genus. To this section belong *N. sylvestris, N. Rusbyi, N. tomentosa* and *N. glutinosa, N. glutinosa,* as well as *N. Tabacum,* show compatbility with the socalled *Rustica* section, but this hypothetical section of the genus is not so well defined as the *Tabacum* section. Only *N. rustica* and *N. paniculata* exhibit very close relationships; with perhaps *N. glauca* standing near. The Petunioides section is not justified from the genetic evidence. There are a number of genetic centers which may possibly be made the basis for various generic subdivisions when our information is more complete.

In conclusion, let me say that I have endeavored to present the genetic work on this genus merely as a matter of historical record. Numerous tentative conclusions and speculative suggestions supported by but few data might be made; but I have endeavored to avoid them.

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REGISTER

Allard, H. A. 264, 283, 295, 298, 302, **308**.
Anastasia, G. E. 292, **308**.
Anderson, E. **309**.
Angeloni, L. **309**.
d'Angremond, A. **309**.
Anonymus **309**.
Arisz, W. H. 299, **309**.

Babcock, E. B., a. R. E. Clausen **309**.
Bateson 301.
Bellair, G. 264, 283, **309**.
Benincasa, M. **309**.
Blakeslee 296.
Brieger, F. G. 249, 263, 269, 272, 278, 279, **309**.
Brieger, F. G., a. A. J. Mangelsdorf 291, **309**.
Brogniart 261.

Castle, W. E. **309**.
Chipman, R. H. a. T. H. Goodspeed 296, **309**.
Chmelař, F., a. J. Simon **309**.
Christoff, M. 247, 249—252, 256, 258—260, 262, 264—266, 268, 271, 273, 274, 286, 298, 303, 307 **309**, **310**.
Chromosome numbers 249.
Clausen, R. E. 248—252, 259, 261—264, 269, 277, **310**.
Clausen, R. E., a. T. H. Goodspeed 273 276, 277, 298, 299, **310**.
Clausen, R. E., a. M. C. Mann 296, **310**.
Clinton, G. P. **310**.
Collins, J. L., a. M. C. Mann 276, **310**.
Comes, O. 244, 263, 264, 281, 292, 301, **310**.
Cytological behaviour 267.

Darwin 288, 291.
David, P. A. 293, **310**.
Datura 299.
Drosophila 299.

East, E. M. 244, 247, 250, 256—266, 269 270, 279, 280, 282, 284—287, 290, 291, 295, 301—304, **310**, **311**.
East, E. M., a. H. K. Hayes 268, 293, 294, 302, **311**.
East, E. M., a. D. F. Jones **311**.
East, E. M., a. A. J. Mangelsdorf 290 **311**.
East, E. M., a. J. B. Park 288, 289, **311**.
Eghis, S. A. 277, **312**.

Fertil hybrids 285.
Focke, W. O. 243, 253, 254, 262, 286, **312**.
Frimmel, F. 302, **312**.

Gaines a. Aase 296.
Garner, W. W. 294, 295, **312**.
Garner, W. W., a. H. A. Allard 295, **312**.
Gärtner, C. F. 243, 245, 246, 253, 254, 258—263, 265, 266, **312**.
Godron, D. A. 243, 245, 254, 286, **312**.
Goetz, E. **312**.
Goodspeed, T. H. 247, 249—252, 259, 267, 273—275, 277, 283, 285, 301, 302, 306, **312**, **313**.
Goodspeed, T. H., a. A. H. Ayres 283, **313**.
Goodspeed T. H., a. R. E. Clausen 246, 258, 261, 262, 265—269, 271, 273, 274, 279, 282—284, 297, 298, 302, 303, **313**.
Goodspeed, Clausen a. Chipman 270, **313**.
Goodspeed, T. H., a. Pirie Davidson **313**.
Goodspeed, T. H., a. J. N. Kendall 283, **313**.
Goodspeed, Mc Cee a. Hodgson **313**.
Goodspeed, T. H., a. A. R. Olson 300. **313**.
Gutierrez, M. E. **313**.

Haig-Thomas, R. 301, **313**. **314**.

HASSELBRING, H. 293, 314.
HAYES, H. K. 293, 295, 314.
HAYES, H. K., a. E. G. BEINHART 295, 314.
HAYES, EAST a. BEINHART 293, 302, 314.
HOFFMANN, 314.
HONING, J. A. 293, 295, 299, 302, 314, 315.
HOUSER, T. 315.
HOWARD, A., a. G. L. C. HOWARD 292, 302, 315.
HOWARD, G. L. C. 292, 302, 315.
HOWARD, G. L. C. a. KASHI RAM 301, 315.
HUNGER, F. W. T. 295, 315.
Hybrids of the pre-Mendelian period 253.
Hybrids of the groups: *N. acuminata* 260, 306; *N. alata* 256, 286, 288; *N. attenuata* 265; *N. Bigelovii* 265; *N. glauca* 261; *N. glutinosa* 261; *N. longiflora* 260, 302; *N. nudicaulis* 266; *N. paniculata* 262; *N. Rusbyi* 263; *N. rustica* 266, 301; *N. suaveolens* 264; *N. sylvestris* 263; *N. Tabacum* 267, 291; *N. tomentosa* 264; *N. trigonophylla* 264.

JEHAN, J. B. 315.
JENSEN, Hj. 294, 303, 315.
JOHNSON, J. 315.
JOHNSON, J., a. R. H. MILTON 315.
JONES, D. F. 294, 296, 315.
JOST 291.

KAJANUS, B. 315.
KARPETSCHENKO 279.
KELANEY, M. A. 298, 299, 315.
KENDALL, J. N. 316.
KLEBS, G. 299, 316.
KÖLREUTER, J. G. 243, 245, 253, 254, 261—263, 316.
KOSTOFF 252, 258—265, 268, 273—275, 307.

LANG, H. 315.
LOCK, R. H. 246, 256—259, 261, 269, 316.
LODEWIJKS, J. A. 294, 295, 303, 316.
LUBEMENKO, V., a. A. PALAMARTCHOUK 316.

MALINOWSKI, E. 264, 283, 316.
MALOCH, W. S., a. F. W. MALOCH 258, 264, 269, 316.
MATHEWSON, E. H. 316.
MILLAN, A. R. 244, 316.
Mutations 295.

NAUDIN, CH. 243, 254, 261, 262, 265, 266, 286, 316.
NIKOLAJEWA, A. 316.

PALM, B. T. 250, 316.
PAOLINI, V. 316.
PARCOF, L. 317.
Partial fertility 279.
Periclinal chimaeras 298.
PREISSECKER, K. 317.

Relationships 307.
REUTHER 317.
RUTTLE, Miss M. 249, 251, 252, 297, 317.
RYBIN, V. A. 250, 277, 317.

SACHS-SKALIŃSKA, M. 287, 288, 317.
SAGERET, A. 243, 317.
SAVELLI, R. 317.
SCHERFFIUS, W. H. 317.
Sections of *Nicotiana* 246.
Selfsterility 288.
SETTCHELL, GOODSPEED a. CLAUSEN 292, 303. 317.
SHAMEL, A. D. 293, 317.
SHAMEL, A. D., a. W. W. COBEY, 293, 318.
SINGLETON, W. R. 271.
SMITH, F. F. 318.
SPLENDORE, A. 308, 318.
STEWART, J. B. 318.

Table of compatibilities 269.
TUKADA, OKADA a. TERAO 318.

VALLEAU, W. D. 318.
VALLEAU, W. D., a. E. J. KINNEY 318.
VRIEND, J. 318.

WELLINGTON, R. 301, 318.
WHITE, O. E. 250, 251, 263, 294, 295, 298, 318.
WINGE, O. 318.
WOYCICKY, Z. 267, 318.

X rays, 300.

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